

Fall 12-16-2017

SYNTHESIS OF UNUSUAL STEROLS FROM MARINE SOURCES

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SYNTHESIS OF UNUSUAL STEROLS FROM MARINE SOURCES

By

Ricardo Joseph

A dissertation

submitted in partial fulfillment of the requirements for the

Doctor of Philosophy Degree

State University of New York

College of Environmental Science and Forestry

Syracuse, New York

December 2017

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ACKNOWLEDGMENTS

First and foremost, I wish to thank my advisor, Prof. José-Luis Giner, for the unbelievable opportunity to work with him and join the Djerassi academic family tree. He is a great mentor, teacher, and by far one of the smartest, most dedicated people I have ever met. I really appreciate all of your help and support.

I would like to thank my steering committee, Prof. Francis Webster and Prof. Gregory Boyer, for their help and guidance through the process. Thank you to Prof. David Kieber and Prof. Joseph Mullins for your time and for serving on my defense committee. I would also like thank Prof. Dibble for his help with enrollment when I first arrived at ESF, Prof. Paul Caluwe for his scientific advice and the opportunity to sit and learn in his class and Dave Kiemle for his indispensable help with the NMRs (300, 600 and 800 MHz). Thank you, Marlene Braun, for always being so nice to me and all the help with lab supplies. Special thank you to Prof. Ivan Gitsov and the Chemistry Department for the funding that allowed me to work and focus on my dissertation over the past summer.

I am also grateful for all the great people and friends that I had to privilege to meet during my time in Syracuse: Scott Bergey, Andrew Hayman, Lise Brown, Eileen Leon, Sara Velardi, Dan Rockefeller, Robert Moesch, Jordan Brinkley, Joy Logan, Dieter Scheibel, Xin Lu, Liang Chen, Zhuyun (Julia) Ye, Seth Beadle, Dr. Ata Pinto and Dr. Jesse Crandall, Dave and Leah Reed. This experience would not be the same without you.

Lastly, I want to thank my mother Madeline and stepfather Pierre Louissaint for the unconditional love, support and encouragement. This would not be possible without you. This is dedicated to my late grandmother and godmother Wilda Joseph.

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LIST OF ABBREVIATIONS

AcOH	Acetic acid
BMS	Borane dimethyl sulfide
BnBr	Benzyl bromide
BTEAC	Benzyltriethylammonium chloride
DCM	Dichloromethane
DMF	N,N-Dimethylformamide
DMP	Dess-Martin periodinane
eq.	Equivalent
EtOAc	Ethyl acetate
EtOH	Ethanol
KtBuO	Potassium tert-butoxide
LAH	Lithium aluminum hydride
mCPBA	m-Chloroperoxybenzoic acid
MeOH	Methanol
MsCl	Methanesulfonyl chloride (mesyl chloride)
n-PrOH	n-Propanol
Ox.	Oxidation
PCC	Pyridinium chlorochromate
rt	Room temperature
tBuOOH	tert-Butyl hydroperoxide

TEA

Triethylamine

TFA

Trifluoroacetic acid

THF

Tetrahydrofuran

Abstract

R. Joseph. Synthesis of Unusual Sterols from Marine Sources, 388 pages, 12 tables, 38 figures, 2017.

The syntheses of some unusual marine sterols are reported. 24-Epiconicasterol, an epimer of the bioactive marine sponge sterol conicasterol, an epimer of the bioactive marine sponge sterol conicasterol was synthesized in 10 steps and a 13% overall yield from ergosterol. Similarly, the 24-epimer of the bioactive sponge sterol theonellasterol, 24-epitheonellasterol, was synthesized from stigmasterol. Pfiesterol, an unusual sterol isolated from the toxic dinoflagellate *Pfiesteria piscicida* and potential biomarker for the species, was synthesized from 16-dehydropregnenolone acetate. 4-Methylcholestane-3 β ,4 β -diol, a synthetic pavlovol, was synthesized from cholestenone in a seven-step sequence and a 38% overall yield. The simple analogs of petrosterol, a bioactive sponge sterol and potential inhibitor of invertebrate sterol metabolism, were synthesized as a mixture from the i-methyl 22-iodide in a six-step sequence and a 43% overall yield. The structures of two novel highly alkylated cyclopropyl sterols from a tropical jewel orchid were elucidated by synthesis.

Methods for sterol synthesis were developed. The use of a sealed reaction vessel shortened the reaction time for the Kirk-Petrow reaction by at least a day. 1-Dodecanethiol was successfully used instead of thiophenol to obtain an odorless product in good yield and with equivalent reactivity. A new method for the oxidation of allylic alcohols via thiyl radicals was developed. Phenyl disulfide and visible light were used to generate the thiyl radicals. This is the first account of the direct involvement of thiyl radicals in the oxidation of allylic alcohols.

This work provided synthetic methods for the aforementioned unusual marine sterols and analogs, which can be used as reference compounds and samples for biological studies. The consumption of unusual marine sterols may affect the growth and development ecologically important marine grazers like copepods.

Keywords: Sterols, marine sterols, biomarkers, unusual sterols, marine sponges, harmful algae, dinoflagellates, phytosterols, pavlovols, conicasterol, organic, synthesis, bioactive sterols, sterol metabolism, thiyl radicals, allylic alcohols, selective oxidation.

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Candidate for the degree of Doctor of Philosophy, December 2017

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Introduction

Sterols are essential lipids present in all eukaryotic cells predominantly as membrane components. Their biosynthesis and function have been extensively studied.¹ Outside of their role in regulating membrane fluidity and permeability, sterols are involved in interactions with other lipid and protein membranes and are precursors to bioactive compounds vital to cellular and developmental processes.² However, the production of sterols is not restricted to eukaryotes. A few species of bacteria are known to synthesize their own sterols.¹ Cholesterol (**1**), the major sterol of vertebrates, is structurally simple compared to sterols from plants, fungi, algae and bacteria.³ Because of their unique and unusual structures, many sterols have been used as biomarkers for marine and terrestrial organisms.⁴⁻⁶ Marine organisms are a major source of structurally diverse sterols and other bioactive compounds. Unusual marine sterols like 24-propylidenecholesterols (**2**) and brevesterol (**3**) have been proposed as biomarkers for algae and dinoflagellate species responsible for harmful algal blooms, respectively (Figure 1.1).⁷⁻¹⁰ Other unusual marine sterols like petrosterol (**4**) and its natural oxygenated analogs were found to exhibit biological activities that include anticancer and antifouling.¹¹⁻¹⁶ Other marine sterols were also found to exhibit antibacterial, antioxidant, anticancer, antidiabetic and anti-hypercholesterolemia properties.¹⁶

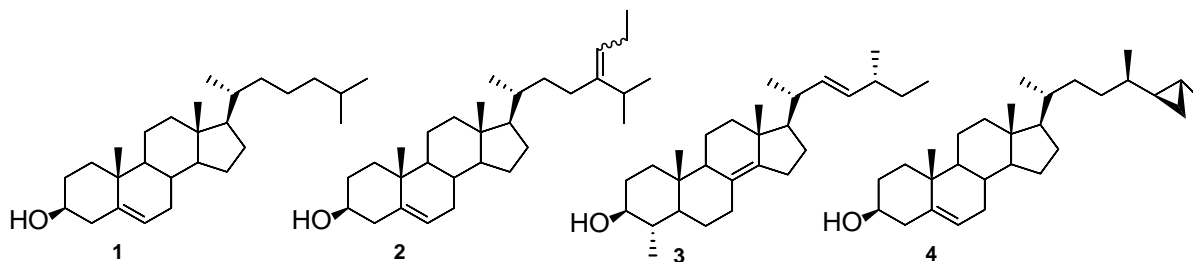


Figure 1.1. Cholesterol and some unusual marine sterols.

Marine sterols with unusual side chains are hypothesized to present in many species as chemical defense against predators.¹⁷ These sterols are thought to interfere with the invertebrate metabolism of phytosterols to cholesterol. Invertebrates are unable to biosynthesize cholesterol, which is a precursor to steroid hormones and is necessary for growth and reproduction.¹⁸ In this work, some unusual marine sterols and analogs were synthesized as part of an investigation to determine their effects on phytosterol metabolism, growth and reproduction in invertebrates. The biological studies, not this described this work, were performed with calanoid copepods (*Acartia hudsonica*, *Eurytemora affinis*, and *Calanus finmarchicus*), a model crustacean, *Artemia salina* by R. Patrick Hassett at Ohio University. Copepods are ecologically important; they are primary consumers of phytoplankton and play a key role in the cycling of elements in the oceans.¹⁹ The 24-epimers of two bioactive sponge sterols were also generated to confirm the stereochemical assignments of the natural products and also provide sample for biological studies. Two unusual highly substituted cyclopropane sterols from a tropical orchid were identified and characterized by synthesis. Methods for sterols synthesis were also developed.

Conicasterol (**5**) and theonellasterol (**6**) are two unusual unsaturated marine sterols isolated as the primary components of the Red Sea sponges *Theonella conica* and *Thenonella swinhoei*, respectively (Figure 1.2).²⁰ Both sterols and their natural analogs were found to be potent and highly selective agonists and antagonists of the orphan nuclear receptors Pregnane X receptor (PXR) and Farnesoid X Receptor (FXR).²¹ FXR, a bile acid nuclear receptor, has been considered a bile acid sensor that has evolved to maintain systemic bile acid homeostasis and protect liver and cells from potentially harmful cellular bile acid overload.²² PXR, a xenobiotic nuclear receptor, plays a vital role in the protection of gastrointestinal tract and liver tissues from xenobiotics and toxic bile acids.²³ The 24-epimer of conicasterol, 24-epiconicasterol (**7**), was synthesized from ergosterol and characterized by NMR. This synthesis would allow the verification of the reported stereochemistry of the 24-methyl of conicasterol and provide samples for biological studies of the synthetic analogue. The C-24 epimer of theonellasterol, 24-

epitheonellasterol (**8**), synthesized from stigmasterol, was also characterized by NMR and compared with the natural product. (Chapter 2).

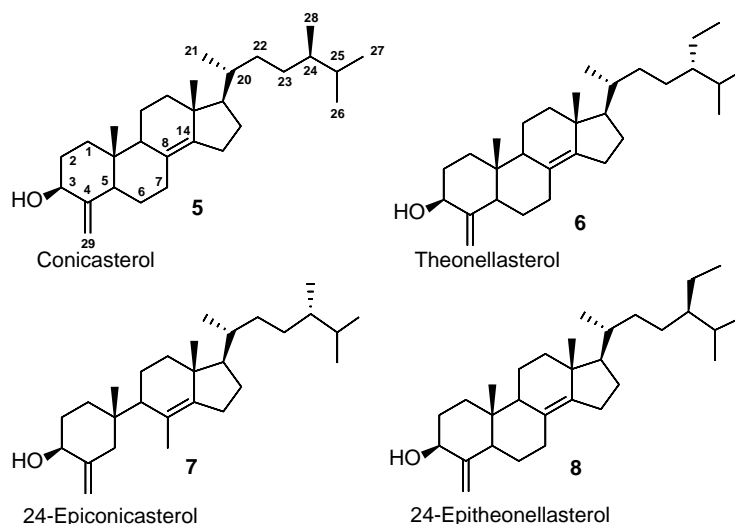


Figure 1.2. Conicasterol, theonellasterol and their C-24 epimers.

Pfiesterol (**9**), a new sterol isolated from two strains of the toxic dinoflagellate *Pfiesteria piscicida*, was identified as a potential biomarker for that species.^{7c} *Pfiesteria* has been linked to massive fish kills and human health issues.²⁴ A published analysis of the sterols in *Pfiesteria piscicida* did not report that compound.²⁵ Pfiesterol was successfully synthesized from 16-dehydropregnenolone acetate (Figure 1.3). Pfiesterol was also synthesized with a 4-¹³C-methyl for metabolic studies in copepods (Chapter 3).

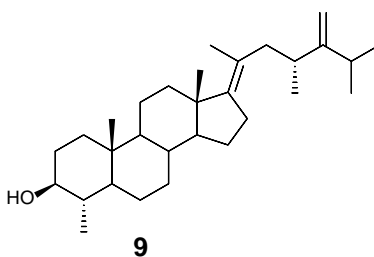


Figure 1.3. Pfiesterol (**9**).

Pavlovols, 3 β ,4 β -dihydroxy-4-methylsterols, are natural sterols that have been exclusively isolated in microalgae belonging to the genera *Pavlova* and *Diacronema*.²⁶⁻²⁹ Pavlovols in cultured *Pavlova* spp. behave as analogs of the natural hormone ecdysone in northern bay scallop (*Argopecten irradians irradians*) larvae.³⁰ 4-Methylcholestane-3 β ,4 β -diol (**10**) was synthesized from cholestenone in a seven-step sequence with a 38% overall yield. Another method to 4-methylcholestane-3 β ,4 β -diol from 4-methylenecholestanol is also described. The synthetic pavlovol (**10**) was characterized by NMR and compared with natural pavlovols (Figure 1.4).²⁶ The synthesis those pavlovols also provided a new substrate for biological studies on bay scallops (Chapter 4).

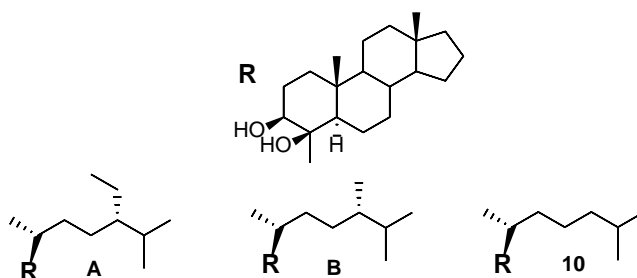
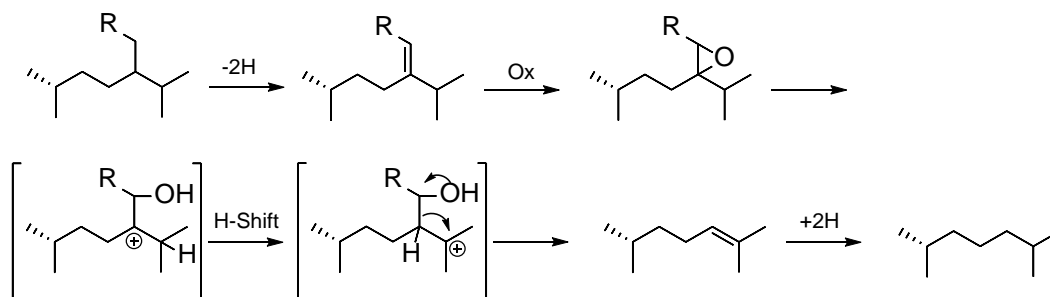


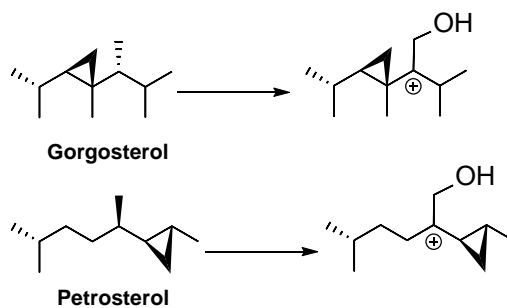
Figure 1.4. Natural pavlovols (**A**) and (**B**) and synthetic pavlovol (**10**).

Many marine invertebrates like crustaceans are unable to biosynthesize sterols and depend on their diet as a source of exogenous sterols like cholesterol.^{31,32} Like in insects, 24-alkyl phytosterols are converted to cholesterol in invertebrates via a well-known dealkylation process (Scheme 1.1).^{31,33} Like gorgosterol, petrosterol (**4**) is a potential inhibitor of invertebrate sterol metabolism. The 24-dealkylation process in both sterols would lead to a highly reactive cyclopropylcarbiny cation capable of disabling the dealkylating enzyme (Scheme 1.2).³⁴ Two simple analogs of petrosterol (**4**) were synthesized as a mixture for samples to study their potential use as inhibitors of invertebrate sterol metabolism. Some potential

metabolic products of the analogs **11**, 24-cyclopropyl-24-methylenechol-5-en-3 β -ol (**12**) and 24,25-epoxy-24-cyclopropyl-24-homochol-5-en-3 β -ol (**13**) were also synthesized (Chapter 5).



Scheme 1.1. Phytosterol dealkylation mechanism in insects.



Scheme 1.2. Carbocation intermediates from the dealkylation of gorgosterol and petrosterol.

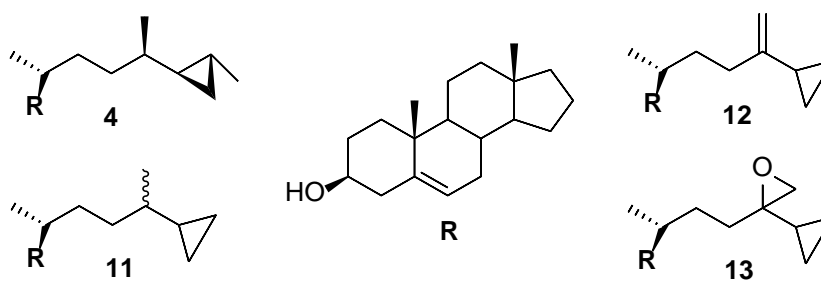


Figure 1.5. Petrosterol **4** and its analogs **11** and potential metabolic products **12** and **13**.

Sterols with side chain cyclopropanes like petrosterol and gorgosterol (Figure 1.5) are unusual in plants. Sterols with cyclopropane-containing side chains have been predominantly observed in marine organisms like dinoflagellates and sea sponges³³. Two novel cyclopropyl sterols (**14** and **15**), isolated in microgram quantities from a tropical jewel orchid, were synthesized and characterized by synthesis (Figure 1.6). To elucidate their stereochemical configurations, all stereoisomers of the candidate structures were synthesized and characterized by 800 MHz NMR. The determination of the stereochemistry of the side chain cyclopropanes was achieved through a series of correlations ultimately resting on (24R)-24-ethyl-24-methylcholestan-3 β -ol, the structure of which ultimately rests on crystallography.³⁵ These correlations included ones with isotopic labeling of prochiral groups (Chapter 6).

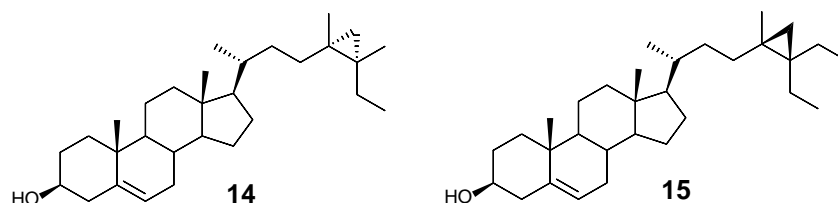
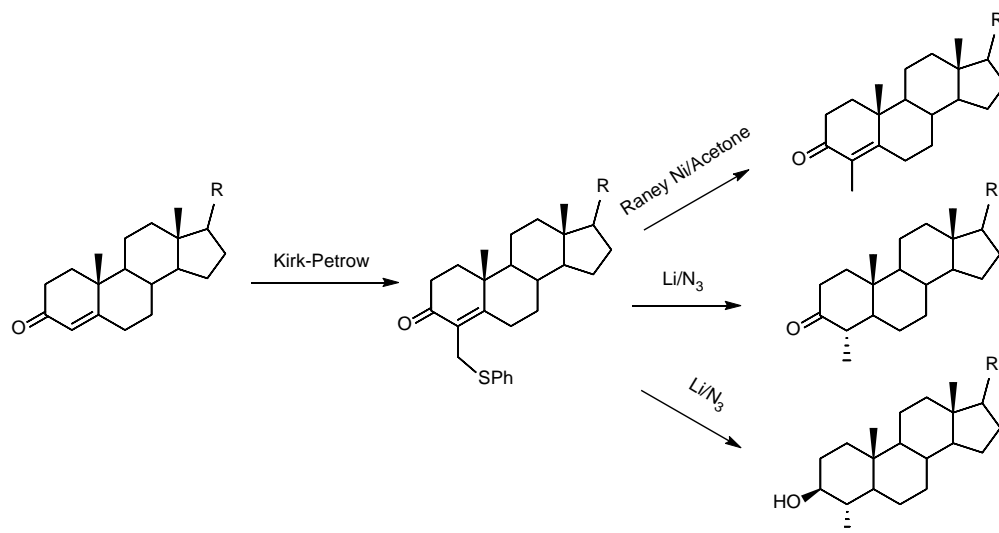


Figure 1.6. Unusual tropical jewel orchid sterols **14** and **15**.

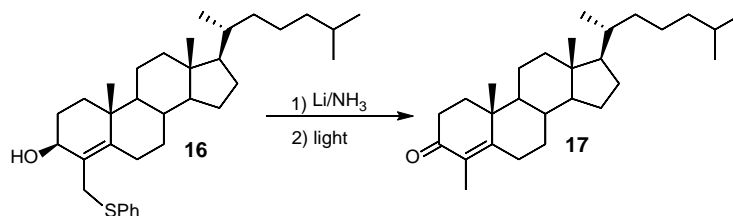
In addition to synthesizing several unusual sterols, two methods for sterol synthesis were developed. The Kirk-Petrow reaction offers a facile and selective method to 4-methyl steroids from 3-oxo- Δ^4 -steroids via a 2-step method (Scheme 1.3).³⁶⁻³⁷ Other reported methods to monomethylate 3-oxo- Δ^4 -steroids at C4 are described as awkward in practice and unselective.^{36,38} A few modifications of the Kirk-Petrow reaction are reported. The use of a sealed reaction vessel reduced the reaction time by at least a day with methanol as the solvent. The use of 1-dodecanethiol instead of thiophenol gave an odorless alkylthiomethylated product in good yield and with comparable reactivity to the typical Kirk-Petrow

reaction product. Cholest-1-en-3-one was used as a substrate for the Kirk-Petrow reaction to probe its mechanism (Chapter 7).



Scheme 1.3. Possible 4-methyl sterols from the Kirk-Petrow reaction.

A new method for the oxidation of allylic alcohols is also reported. A solution of phenyl disulfide in hexanes/EtOAc was found to oxidize allylic alcohols to their corresponding ketones and aldehydes under visible light. This new oxidation method is selective and works very well for 3-hydroxy- Δ^4 -sterols (Scheme 1.4). This is the first account of the involvement of thiyl radicals in the oxidation of allylic alcohols. A few non-steroidal, low molecular-weight allylic and benzylic alcohols were also oxidized with the reaction in CDCl_3 as a solvent (Chapter 8).



Scheme 1.4. Desulfurization and oxidation of compound **16** to **17**.

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CHAPTER 2

Synthesis and Characterization of 24-Epiconicasterol

Abstract

The synthesis and characterization of the 24-epimer of conicasterol from ergosterol is reported. The key step in this synthetic sequence involves the PtO_2 catalyzed hydrogenation of $(3\beta,5\alpha,8\alpha,22\text{E})$ -epidioxyergosta-6,22-dien-3-ol to $(3\beta,5\alpha)$ -ergost-8(14)-en-3,5-diol. In one step, this reaction reduces the side chain and Δ^6 double bonds and sets the nuclear $\Delta^8(14)$ through the reduction of endo-peroxide and subsequent elimination of the intermediate C8 hydroxyl. The synthesis was achieved in a 10-step sequence and a 13% overall yield. The spectral data for the 24-epimer of theonellasterol and 24-desmethylconicasterol are also reported. This synthesis allowed the verification of the stereochemical assignments of the natural products and provided samples for the biological studies of the synthetic 24-epimers as potential ligands of the nuclear receptors PXR and FXR.

2.1 Introduction

Conicasterol (**1**) and theonellasterol (**2**) are two unusual unsaturated marine sterols isolated as the primary components of the Red Sea sponges *Theonella conica* and *Thenonella swinhoei*, respectively. They were the first reported sterols to simultaneously contain the 4-methylene and $\Delta^{8(14)}$ functionalities (Figure 2.1).¹ The side chain stereochemistry for conicasterol and theonellasterol were elucidated via ^1H

NMR comparison with two known sterols with similar side chains.¹ Several sterols derived from the oxidation of these sterols have been isolated.² Conicasterol, theonellasterol and their natural analogs are potent and highly selective agonists and antagonists of the orphan nuclear receptors Pregnane X receptor (PXR) and Farnesoid X Receptor (FXR).³ FXR, a bile acid nuclear receptor, has been considered a bile acid sensor that has evolved to maintain systemic bile acid homeostasis and protect liver and cells from potentially harmful cellular bile acid overload.⁴ PXR, a xenobiotic nuclear receptor, plays a vital role in the protection of gastrointestinal tract and liver tissues from xenobiotics and toxic bile acids.⁵ It is also expressed at a lower level in the kidney and ovary tissues and its dysfunction is associated with immune disorders including primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), and inflammatory bowel diseases (IBDs), ulcerative colitis and Crohn's disease.⁶ The observed biological activities of these limited and not so readily accessible natural products make it important to synthesize analogs that may share their biological properties and potential pharmacological interests.

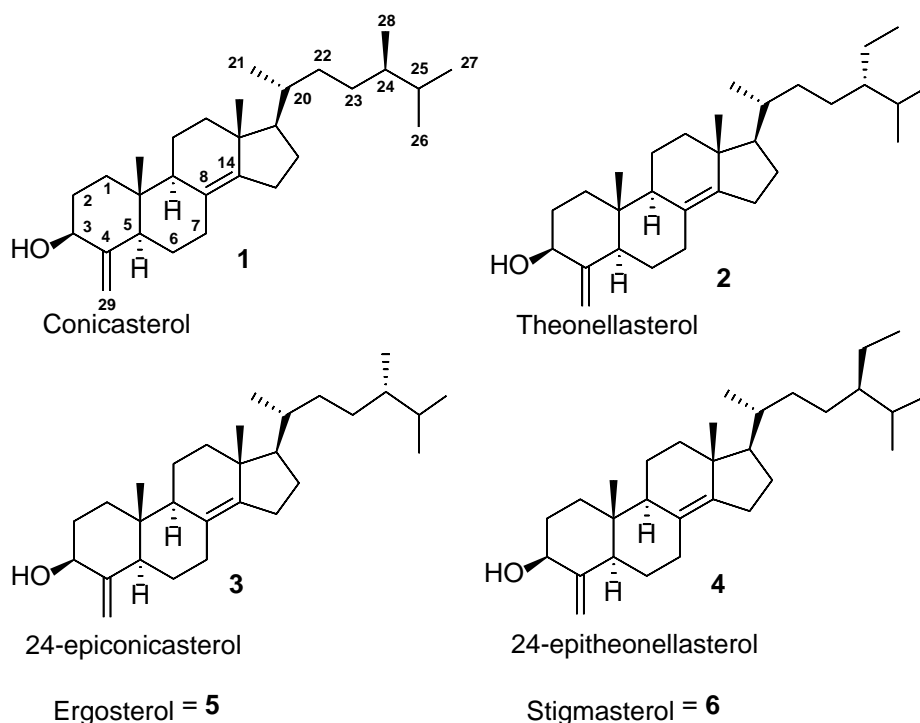
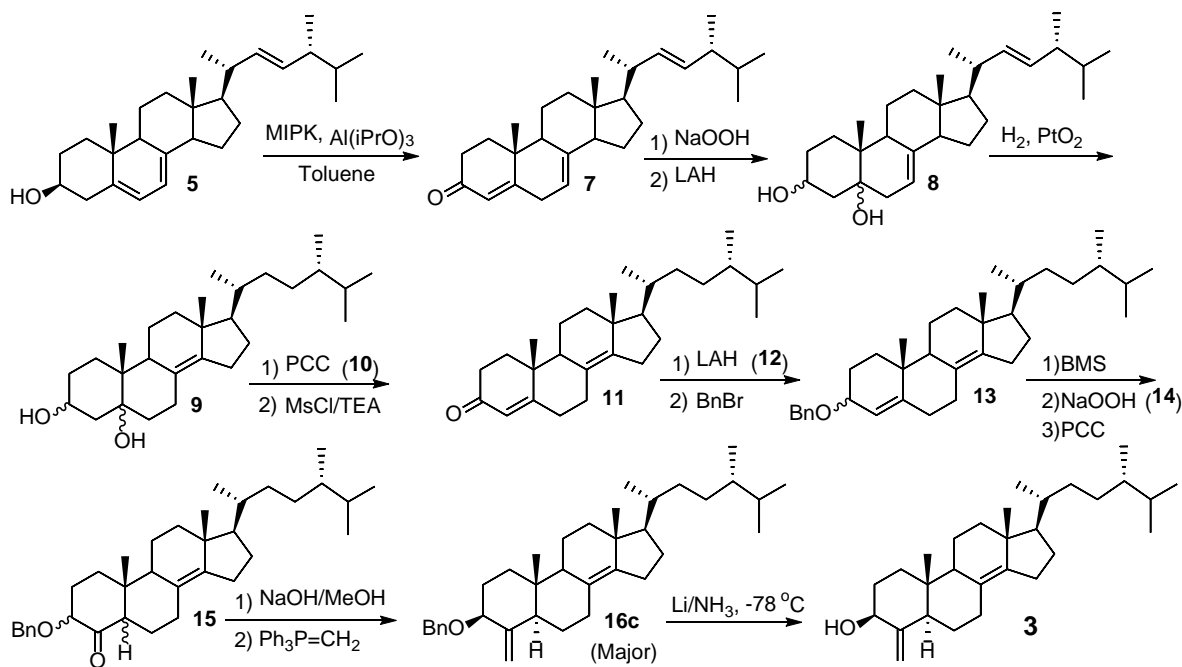


Figure 2.1. Conicasterol, theonellasterol and their C-24 epimers.

Herein, the synthesis of the 24-epimer of conicasterol and its NMR spectral data are reported. This synthesis is important as it allows the verification of the reported stereo chemistry of the 24-methyl of conicasterol and provides samples for biological studies of the synthetic analogue. Spectral data for the C24 epimer of theonellasterol, 24R-theonellasterol (**4**), synthesized from stigmasterol (**6**) are also described. The ^1H NMR spectrum of 24-desmethylconicasterol is also reported.

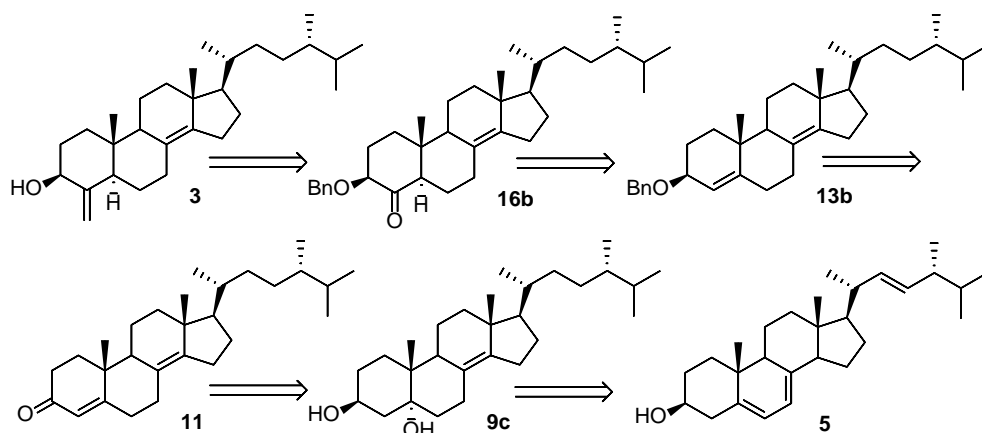
2.2 Results and Discussion

An original approach to the synthesis of 24-epiconicasterol from ergosterol is described in Scheme 2.1. The Δ^7 was carried through the first of two steps the sequence before converting it into the desired $\Delta^{8(14)}$ by catalytic hydrogenation.⁷ The desired product, 24-epiconicasterol (**3**), was obtained at the end of the sequence; however, this approach was considered ineffective because of the poor yield and many unidentified side products obtained from the hydrogenation/ Δ^7 isomerization step.



Scheme 2.1. Synthesis of 24-epiconicasterol from ergosterol.

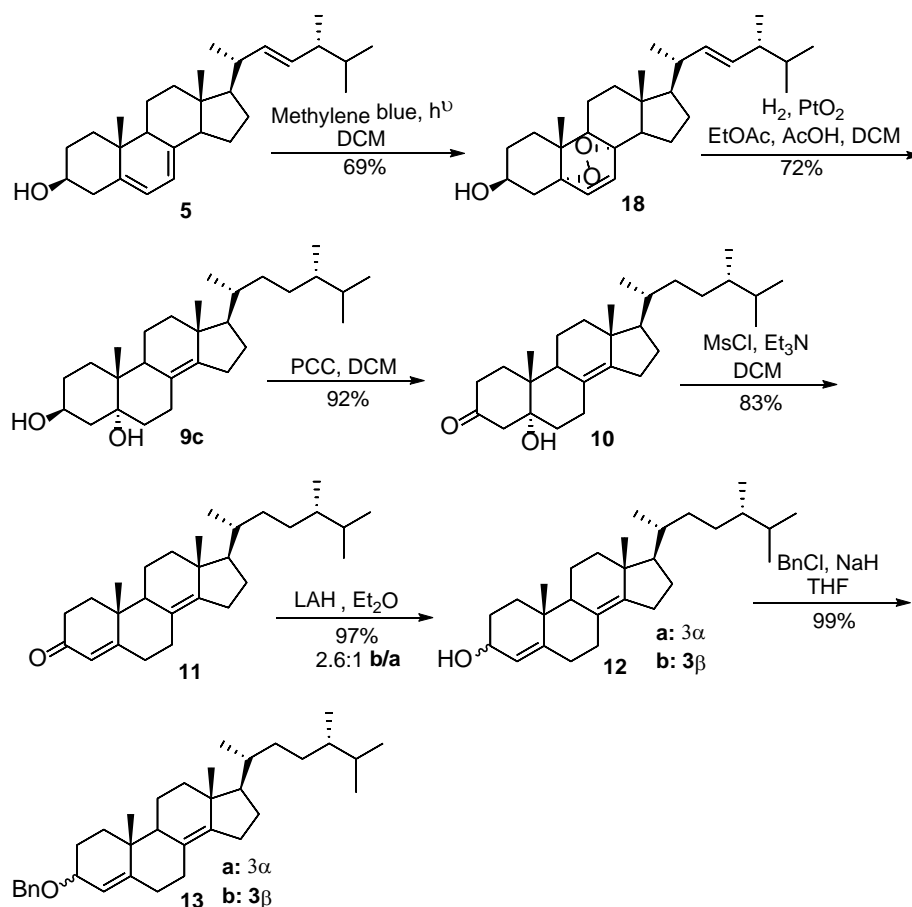
A more efficient method was developed to synthesize 24-epiconicasterol (**3**) from ergosterol (**5**). The first four steps in Scheme 2.1 were replaced just with two steps to achieve the $\Delta^{8(14)}$ -diol intermediate (**8**). A retrosynthetic analysis is displayed in Scheme 2.2.



Scheme 2.2. Retrosynthetic analysis of 24-epiconicasterol (**3**).

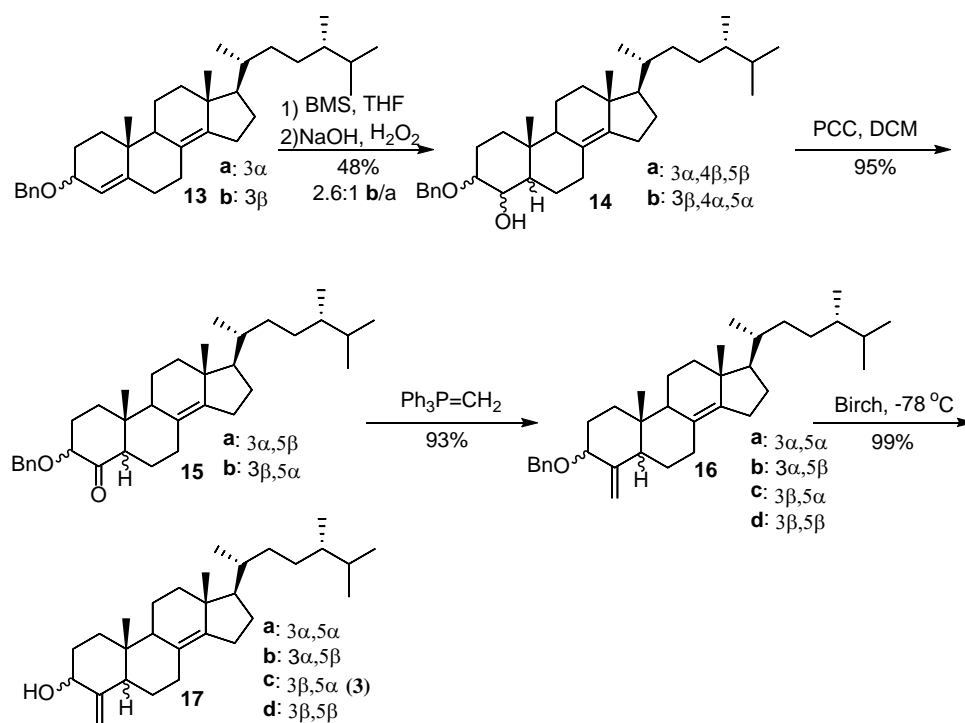
The synthesis of 24-epiconicasterol began with the dye-activated photooxygenation of ergosterol (**5**) to afford the endoperoxide **18** as the major product (Scheme 2.3).⁸ The PtO_2/AcOH catalyzed hydrogenation of compound **18** gave the unsaturated diol **9c**.⁹ The 3-OAc of compound **9c** and (3 β ,5 α)-ergost-8(14)-en-3-ol (not displayed in the schemes) were also isolated as side products. (3 β ,5 α)-Ergost-8(14)-en-3-ol was likely obtained by the elimination of the 5 α -OH in compound **9c** followed by subsequent hydrogenation of the resulting double bond. The 3-OAc was easily converted to compound **9c** via transesterification with methanolic NaOH. Compound **9c** was then oxidized with PCC to the unsaturated keto-alcohol **10**.¹⁰ The dienone **11** was generated in good yield upon treatment of intermediate **10** with MsCl and Et_3N .¹¹ The treatment of compound **11** with LAH gave a hard to separate mixture of the dienol **12** with a 2.6 to 1 ratio favoring the desired isomer **12b**. The dienol **12b** was considered the

desired diastereomer as a hydroboration test with a similar benzylated substrate 3 β -benzyloxy-cholest-4-ene (**21b**) gave only one product (3 β ,4 α ,5 α)-3-benzyloxy-cholesta-3,4-diol (**22b**) with the desired stereochemistry at positions 3 and 5 (Scheme 2.5). The dienol mixture was benzylated with benzyl bromide and the product **13** was converted to **14** via a hydroboration-oxidation reaction (Scheme 2.4). A modest 48% yield was observed for the hydroboration reaction as some more polar undesired side products were generated during the oxidation step of the reaction. Gentle heating during the oxidation step was found to help improve the reaction yield.



Scheme 2.3. Synthesis of intermediate **13** from ergosterol.

Since a separation via flash chromatography was still difficult on a large scale at that stage the mixture of **14** was oxidized with PCC in a high yield (95%) to afford **15** as a mixture with a 2.6 to 1 ratio that favored the desired diastereomer **14b**. That product mixture was consistent with the ratio obtained from LAH reduction of the enone **11** and the isolated hydroboration products. The ketone mixture was then methylenated via a Wittig reaction to get a mixture of 4 products (**16**); compound **16c** was the major and desired product. The different components of the mixture were identified by deprotecting a sample of the crude product mixture and matching each product with previously identified sterols in our lab. The yield and ^1H NMR data of the products are listed in Table 2.1. The product ratio of the Wittig reaction suggests that an epimerization of the starting ketones occurred before methylenation took place. This was verified by treating the intermediates **15a** and **15b** individually with 5% methanolic NaOH and refluxing the mixture for 1 h. Ketone **15b** appeared to be stable to those conditions or thermodynamically favored as no other products were recovered. However, ketone **15a** gave a mixture of products that included intermediate **15b**. Energy minimization calculations (MM2) with Chem3D¹² of the ketones **15a** and **15b** revealed that the major product of the methylenation (**16c**) (79%) was generated from the higher-energy intermediate (**15b**), 56.2 kcal/mol, (Table 2.1). The lower-energy ketone **15a**, 52.4 kcal/mol, was responsible for only 7% of methylenation products, compound **16b**. The final ratio of the methylenation products was likely more dependent on the energy of their transition states rather than that of their corresponding starting ketones based on MM2 calculation data.



Scheme 2.4. Synthesis of 24-epiconicasterol from intermediate **13**.

Table 2.1. Ergost-8(14)-en-3-benzyloxy-4-methylene products.

Product	% yield	4-Methylene ¹ H (ppm)	Benzyl ¹ H (ppm)
3 α 5 α (16a)	6	4.934, 4.737	4.481 (d, <i>J</i> = 12.1 Hz), 4.281 (d, <i>J</i> = 12.1 Hz)
3 α 5 β (16b)	7	5.439, 5.002	4.657 (d, <i>J</i> = 12.2 Hz), 4.606 (d, <i>J</i> = 12.2 Hz)
3 β 5 α (16c)	79	5.217, 4.650	4.701 (d, <i>J</i> = 12.1 Hz), 4.569 (d, <i>J</i> = 12.1 Hz)
3 β 5 β (16d)	8	5.084 (2H)	4.487 (d, <i>J</i> = 12.2 Hz), 4.273 (d, <i>J</i> = 12.2 Hz)

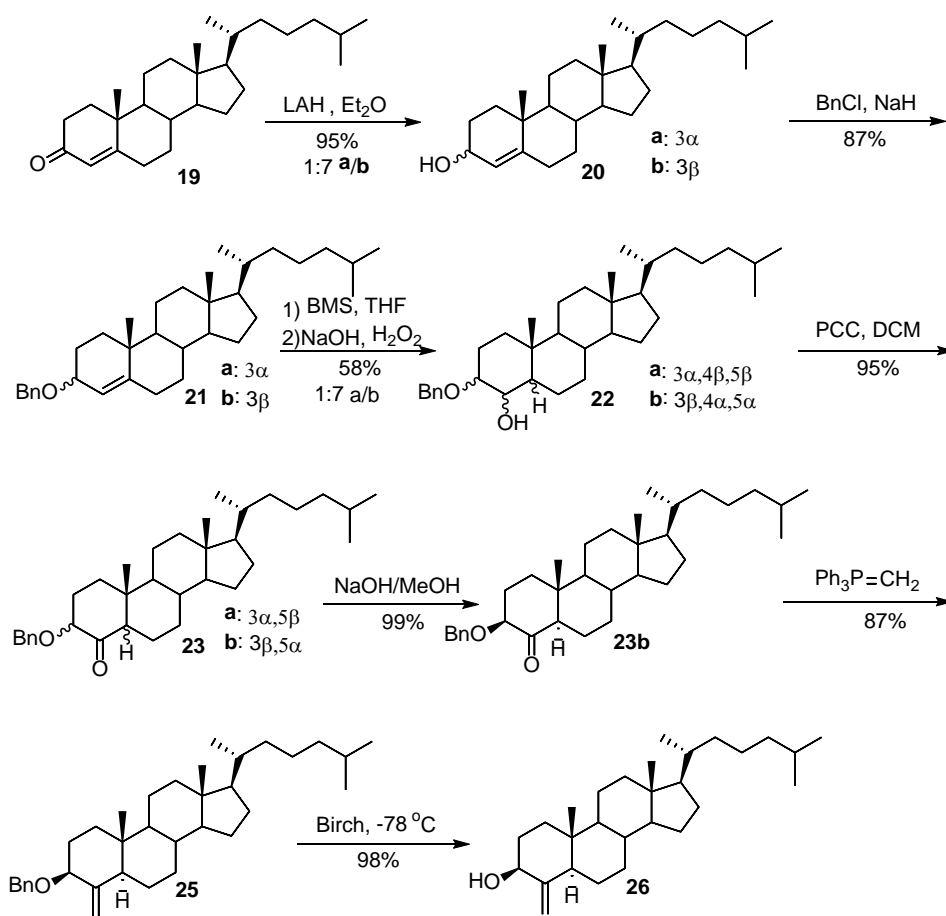
The Wittig reaction was also attempted with the ketones **15a** and **15b** with an ethoxymethyl protecting group instead of the benzyl group. No improvement in yield was observed and the stability of each starting ketone was relatively the same. A 7:1 (3 α 5 β /3 β 5 α) mixture of the Δ^0 -cholesterol analogs of ketone **11** generated from cholest-4-en-3-one (**19**), 3-benzyloxy-cholestan-4-one **24**, was used as a model to test the methylation reaction (Scheme 2.5). Unlike ketone mixture **11**, intermediates **23** easily and

cleanly epimerized to the desired ketone **23b** when refluxed in methanolic NaOH. The methylenation of the ketone mixture **23** gave a similar mixture of the 4-methylene products observed with ketone **15**. The same reaction with the pure ketone **23b** also gave a mixture. However, an improved yield of the desired product **22** (87%) was obtained compared to the 79% yield observed with the ketone mixture. The Wittig reaction with the pure ketone suggested that some epimerization took place before formation of the Wittig products.

To achieve the final product **17**, the Wittig product mixture **16** was debenzylated in near quantitative yield with a dissolving metal reduction (Li/NH_3)¹³ at $-78\text{ }^\circ\text{C}$. The traditional dissolving metal reduction of the benzyl ether mixture **16** without the $-78\text{ }^\circ\text{C}$ bath gave some over-reduced side products by also reducing the 4-methylene. The four products were separated by HPLC and characterized by NMR. The isolation of compound **16c**, the more polar and desired product, from the Wittig product mixture using preparative TLC also provided a pure sample of 24-epiconicasterol **3** after the $-78\text{ }^\circ\text{C}$ dissolving metal (Li/NH_3) reduction of the benzyl ether. Compound **25** was also debenzylated via the method described above to afford 4-methylenecholesterol¹⁴ (**26**).

The NMR data of 24-epiconicasterol (**3**) was compared to the conicasterol NMR data reported by Kho et al.¹ The ^1H NMR assignments for C26, C27 and C28 were in disaccord. Since C26 and C27 are interchangeable by HSQC, HMBC and COSY analyses, C28 was chosen as the focus of the comparison (Table 2.2). Campesterol (24R-24-methylcholesterol) and 22-dehydrobrassicasterol (24S-24-methylcholesterol), two natural sterols with similar side chains, were used as models to compare the C28 ^1H chemical shifts. The Δ value of the C28 ^1H chemical shifts of 22-dehydrobrassicasterol (0.787 ppm) and **3** (0.788 ppm) was only -1 ppb. A similar Δ value, -1 ppb, was observed by comparing the C28 ^1H chemical shift of campesterol (0.776 ppm) with the signal reported by Kho et al¹ as C26 (0.777 ppm), most likely a missassignment. Their ^1H NMR assignment for C28, 0.851 ppm (d, $J = 7.0\text{ Hz}$), matched

the signal that was assigned as C26/C27 0.854 ppm (d, $J = 6.9$ Hz) by HSQC-DEPT and HMBC. A Δ value of -11 ppb was observed when the C28 ^1H signals of conicasterol and 24-epiconicasterol were directly compared. The ^{13}C NMR spectral data of compound **3** matched the nuclear carbon signals of the natural product within Δ values of -120 to 60 ppb (Table 2.3). Some of the reported carbons were also missassigned (C1, C6, C15 and C16). The side chain carbons had higher Δ values as expected ranging from -840 ppb (C25) and +260 ppb (C20). The direct comparison of the ^{13}C NMR data of conicasterol¹ and **3** allowed an easy assignment of the sp^2 carbons.



Scheme 2.5. Synthesis of (3β,5α)-4-methylene-cholestan-3-ol from cholest-4-en-3-one.

Table 2.2. ^1H NMR comparison of conicasterol **1** and its 24-epimer **3**. a) Literature ^1H NMR chemical shifts in ppm. b) Measured ^1H NMR chemical shifts in ppm.

Compound	C18	C19	C28	=CH ₂	=CH ₂
1 ^a	0.836	0.588	0.851	5.073	4.631
3 ^b	0.838	0.592	0.786	5.076	4.633
Δ (ppb)	-2	-4	+65	-3	-2

The ^1H and ^{13}C NMR data of the 24-epimer of theonellasterol synthesized from stigmasterol via a similar method are reported in the experimental section. The ^1H NMR data of theonellasterol and 24-epitheonellasterol are compared in Table 2.4. The Δ values for the side chain ^1H signals varied from -4 to +5 ppb. C29 was the only carbon with a positive Δ value, +5 ppb. A positive Δ value was also observed between the C28 ^1H signals of conicasterol and its 24-epimer. The ^1H NMR data of 24R-24-ethylcholesterol and 24S-24-ethylcholesterol were compared to make sense of the small differences in chemical shift ($\Delta\delta$) observed between the side chain signals of theonellasterol and its 24-epimer. A +8 ppb $\Delta\delta$ value was observed for the C29 methyl ^1H NMR signals of the two 24-ethylcholesterol isomers. This small $\Delta\delta$ value was similar that observed between the C29 ^1H NMR signals of theonellasterol and its 24-epimer. A more useful comparison was the $\Delta\delta$ between C29 and the most upfield methyl doublet (C26/C27) of the side chain isopropyl group. Using this comparison, a 32 ppb $\Delta\delta$ value was observed for both 24-epiconicasterol and 24R-24-ethylcholesterol, whereas $\Delta\delta$ values of 43 and 45 ppb were observed between the same the side chain ^1H NMR signals for theonellasterol and 24S-24-ethylcholesterol, respectively. The similarity between these $\Delta\delta$ values between the two 24R and the two 24S sterols confirmed the stereochemical difference between the theonellasterol and its synthetic 24-epimer. The ^1H NMR data for 24-desmethylconicasterol, a synthetic analogue of those marine sterols, is also reported in the experimental section.

Table 2.3. ^{13}C NMR data for conicasterol and 24-epiconicasterol. Bolded carbon numbers are different from reported assignments¹. */+ Assignments may be reversed.

Conicasterol ¹		24-Epiconicasterol		Δ -value (ppb)
Carbon #	(ppm)	Carbon #	(ppm)	
1	34.55	20	34.81	260
2	33.16	2	33.21	50
3	73.37	3	73.40	30
4	153.11	4	153.17	60
⁺ 5	49.49	5	49.48	-10
6	27.05	16	27.04	-10
7	29.37	7	29.38	10
8	125.69	8	125.69	0
⁺ 9	49.28	9	49.27	-10
10	40.00	10	40.01	10
11	20.45	11	20.48	30
*12	37.38	12	37.35	-30
13	42.76	13	42.75	-10
14	142.93	14	142.94	10
15	24.68	6	24.68	0
16	25.80	15	25.82	20
17	56.90	17	56.78	-120
18	18.22	18	18.20	-20
19	13.20	19	13.20	0
20	36.77	1	36.75	-20
21	19.10	21	19.28	180
22	33.55	22	33.56	10
23	30.21	23	30.43	220
24	38.95	24	39.13	180
25	32.39	25	31.55	-840
*26	20.21	26/27	20.44	230
27	18.22	26/27	17.65	-570
28	15.39	28	15.45	60
29	102.82	29	102.77	-50

Table 2.4. ^1H NMR comparison of theonellasterol **2** and its 24-epimer **4**. a) Literature¹ ^1H NMR chemical shifts in ppm. b) Measured ^1H NMR chemical shifts in ppm.

Compound	C18	C19	C29	=CH ₂	=CH ₂
2 ^a	0.836	0.588	0.856	5.073	4.629
4 ^b	0.840	0.591	0.851	5.075	4.632
Δ (ppb)	-4	-3	+5	-2	-3

2.3 Conclusions

Ultimately, the synthesis of the 24-epiconicasterol was completed from ergosterol in a 10-step sequence and a 13% overall yield. The NMR data for 24-epiconicasterol confirmed the reported stereochemistry at C24 (24R) for the natural product conicasterol. High Δ values were observed between the side chain ^{13}C NMR signals of conicasterol and 24-epiconicasterol and a +65 ppb difference was measured between their C28 ^1H signals. A +5 ppb Δ value was also measured between the C29 ^1H signals of 24-epitheonellasterol and theonellasterol to also confirm the reported stereochemistry of the natural sterol. We were hesitant to confirm the stereochemistry of theonellasterol because of the relatively small $\Delta\delta$ value measured between its C29 ^1H NMR signal and that of its 24-epimer. However, comparing the larger $\Delta\delta$ values between C29 and the most upfield methyl doublet (C26/C27) ^1H signals of the side chain isopropyl groups allowed correlations to be made between theonellasterol and 24S-24-ethhylcholesterol, and 24-epitheonellasterol and 24S-24-ethhylcholesterol that confirmed the stereochemical assignment by Kho et al¹.

This synthesis also provided samples for biological studies. Natural analogs of both, conicasterol and theonellasterol have been identified as ligands to the FXR and PXR nuclear receptor.³ Bioactivity of the synthetic epimers would be of great significance as they could be an alternative source of ligands with pharmaceutical interests for the nuclear receptors PXR and FXR.

2.4 Experimental

General Procedures. NMR spectra were obtained in CDCl₃ on a Bruker 600 MHz spectrometer (¹H at 600 MHz and ¹³C at 151 MHz). HPLC was performed using a Waters 6000A pump, Waters 410 differential refractometer, and two Altex Ultrasphere ODS columns (5 μm, 10 x 250 mm) in series at a flow rate of 3 mL/min with MeOH. Preparative TLC were done with Sorbent Technologies (SORBTECH) silica gel HL TLC plates (20 x 20 cm², 250 μm thick with glass backed support). All solvents were from commercial sources.

(3β,5α,8α,22E)-Epidioxyergosta-6,22-dien-3-ol (18). A solution of ergosterol (1.04 g, 2.62 mmol) and methylene blue (0.01 g) in 80 mL of CH₂Cl₂ was stirred in an open flask at 0 °C. The solution was irradiated with a 90 W tungsten lamp placed 15 cm away, at a 45-degree angle to the surface of the liquid. After 3.5 h, the mixture was filtered through silica gel and eluted with 1:1 hexanes/EtOAc. The crude product was purified by silica gel chromatography with 2:1-1:1 hexanes/EtOAc to give 0.77 g of **6** as a white solid (69% yield). ¹H NMR (600 MHz): 6.502 (1H, d, *J* = 8.5 Hz), 6.240 (1H, d, *J* = 8.5 Hz), 5.223 (1H, dd, *J* = 7.7, 15.3 Hz), 5.147 (1H, dd, *J* = 8.4, 15.3 Hz), 3.970 (1H, tt, *J* = 5.0, 11.5 Hz), 2.109 (1H, ddd, *J* = 1.9, 5.0, 13.8 Hz), 1.693 (1H, dt, *J* = 3.5, 13.4 Hz), 0.999 (3H, d, *J* = 6.7 Hz), 0.909 (3H, d, *J* = 6.9 Hz), 0.884 (3H, s), 0.834 (3H, d, *J* = 6.8 Hz), 0.817 (3H, d, *J* = 6.8 Hz), 0.817 (3H, s).

(3 β ,5 α)-Ergost-8(14)-ene-3,5-diol (9c). A solution of **18** (468.3 mg, 1.12 mmol) and PtO₂ (141.2 mg) in 10 mL of 4:2:1 EtOAc/acetic acid/dichloromethane was stirred at atmospheric pressure under a hydrogen atmosphere. The reaction was monitored by NMR until completion (5 days). After filtration, the solvent was removed under reduced pressure and the product was purified by silica gel chromatography with 2:1 hexanes/EtOAc to yield 737.4 mg of **7** as an off-white solid, and 16.5 mg of ergost-8(14)-en-3 β -ol. An early fraction containing the 3-acetates of both compounds was deacetylated with 10% methanolic NaOH at reflux and purified by chromatography to give an additional 29.1 mg of **9c** (72% total yield) and 16.5 mg of ergost-8(14)-en-3 β -ol. **(3 β ,5 α)-Ergost-8(14)-ene-3,5-diol:** ¹H NMR (600 MHz): 4.082 (1H, m), 2.149 – 2.063 (1H, m), 1.996-1.929 (1H, m), 1.681-1.738 (1H, m), 0.935 (3H, d, *J* = 6.6 Hz), 0.875 (3H, s), 0.856 (3H, d, *J* = 6.8 Hz), 0.849 (3H, s), 0.786 (3H, d, *J* = 6.8 Hz), 0.783 (3H, d, *J* = 6.8 Hz). ¹³C NMR (151 MHz): 143.4, 125.1, 75.2, 67.2, 56.7, 44.2, 42.8, 41.2, 40.1, 39.1, 37.4, 34.9, 34.6, 33.5, 31.6, 31.2, 30.7, 30.5, 27.0, 25.8, 24.2, 20.5, 20.1, 19.2, 18.3, 17.7, 17.4, 15.5. **(3 β)-Ergost-8(14)-en-3-ol:** ¹H NMR (600 MHz): 3.619(1H, m), 0.935 (3H, d, *J* = 6.7 Hz), 0.855 (3H, d, *J* = 6.8 Hz), 0.841 (3H, s), 0.785 (3H, d, *J* = 6.9 Hz), 0.783 (3H, d, *J* = 6.9 Hz), 0.690 (3H, s).

(5 α)-Ergost-8(14)-en-5-hydroxy-3-one (10). A solution of **7** (268 mg, 0.64 mmol) and PCC (518.3 mg, 2.4 mmol) in 10 mL of CH₂Cl₂ was stirred for 1 h. Hexanes (20 mL) was added to reaction and the resulting mixture was filtered through silica gel and eluted with 2:1 hexanes/EtOAc to yield 244.6 mg (92% yield) of desired product as white solid. ¹H NMR (600 MHz): 2.696 (1H, d, *J* = 15.1 Hz), 2.151 (1H, d, *J* = 15.4 Hz), 1.077 (3H, s), 0.946 (3H, d, *J* = 6.6 Hz), 0.885 (3H, s), 0.860 (3H, d, *J* = 6.8 Hz), 0.789 (3H, d, *J* = 6.8 Hz). ¹³C NMR (151 MHz): 210.8, 143.9, 124.5, 77.8, 56.8, 52.0, 42.8, 41.3, 40.6, 39.1, 38.1, 37.3, 34.8, 34.6, 33.6, 30.5, 31.6, 30.5, 27.0, 25.9, 24.4, 20.5, 20.1, 19.3, 18.4, 17.7, 16.7, 15.5.

Ergosta-4,8(14)-dien-3-one (11). Triethylamine (2 mL) was added to a stirred solution of **10** (242.8 mg, 0.61mmol) in 8 mL of anhydrous CH₂Cl₂ at 0 °C. After 10 min, 0.8 mL of MsCl in 1 mL of anhydrous CH₂Cl₂ was added dropwise and the resulting mixture was stirred at that temperature for 30 min. The

reaction was quenched with saturated sodium bicarbonate and extracted with 4:1 hexanes/EtOAc. The organic layer was dried over Na₂SO₄ and evaporated under reduced pressure. The crude product was purified via silica gel chromatography with 19:1 hexanes/EtOAc to yield 194.2 mg of an off-white solid (83%). ¹H NMR (600 MHz): 5.756 (1H, br s), 2.525 (1H, ddd, *J* = 2.8, 5.9, 13.9 Hz), 2.061-1.984 (2H, m), 1.088 (3H, s), 0.949 (3H, d, *J* = 6.7 Hz), 0.902 (3H, s), 0.858 (3H, d, *J* = 6.9 Hz), 0.788 (3H, d, *J* = 6.9 Hz). ¹³C NMR (151 MHz): 199.6, 171.6, 144.9, 123.9, 123.3, 56.7, 47.4, 42.9, 40.0, 39.1, 37.3, 34.8, 34.5, 34.1, 33.5, 32.6, 31.6, 30.4, 29.7, 27.0, 25.9, 20.5, 19.7, 19.2, 18.5, 18.3, 16.6, 15.5.

Ergosta-4,8(14)-dien-3-ol (11). A solution of LAH (89.3 mg, 2.35 mmol) in 2 mL of anhydrous ether was added dropwise to a stirred mixture of **10** (194.2 mg, 0.490 mmol) in 4 mL of anhydrous ether. After 30 min, the reaction was quenched by slow addition of 5 mL of brine. The resulting mixture was diluted with 10% hydrochloric acid until acidic and extracted with 4:1 hexanes/EtOAc. The organic layer was washed with saturated sodium bicarbonate, dried over Na₂SO₄, filtered through silica and evaporated under reduced pressure to give 189.3 mg (97%) of a 2.6:1 Ergosta-4,8(14)-dien-3β-ol (10b)/Ergosta-4,8(14)-dien-3α-ol (10a) mixture. A sample of the product was separated via HPLC for NMR analysis of the two products. **11a** ¹H NMR (600 MHz): 5.463 (1H, dd, *J* = 1.3, 4.3 Hz), 4.116 (1H, m), 2.448 (1H, ddd, *J* = 2.0, 5.6, 13.5 Hz), 2.056 (1H, ddd, *J* = 1.9, 5.5, 13.5 Hz), 1.970 (1H, dt, *J* = 3.5, 12.5 Hz), 0.945 (3H, d, *J* = 6.6 Hz), 0.900 (3H, s), 0.876 (3H, s), 0.859 (3H, d, *J* = 6.8 Hz), 0.789 (3H, d, *J* = 6.8 Hz), 0.787 (3H, d, *J* = 6.8 Hz). **11b** ¹H NMR (600 MHz): 5.330 (1H, br s), 4.189 (1H, m), 2.45 (1H, ddd, *J* = 2.0, 5.5, 13.8 Hz), 2.047 (1H, ddd, *J* = 1.9, 5.5, 13.4 Hz), 0.965 (3H, s), 0.941 (3H, d, *J* = 6.7 Hz), 0.871 (3H, s), 0.857 (3H, d, *J* = 6.9 Hz), 0.786 (3H, d, *J* = 6.8 Hz), 0.785 (3H, d, *J* = 6.8 Hz).

3-Benzoyloxy-ergosta-4,8(14)-diene (12). Benzyl bromide (70 μL, 0.6 mmol) was added to a solution of **10** (162 mg, 0.4 mmol) dissolved in 5 mL of dry THF in a 25-mL flask equipped with a reflux condenser. NaH (102.1 mg), washed in hexanes, was transferred in 3 mL of anhydrous THF to the reaction mixture before heating the resulting solution at reflux under a N₂ atmosphere. After 16 h, the reaction was cooled

to rt, filtered through silica gel and eluted with 4:1 hexanes/EtOAc. The crude product was evaporated under reduced pressure. Flash chromatography using 79:1-39:1 hexanes/EtOAc gave (196.8 mg, 99%) of an inseparable 2.6/1 β/α mixture of **12** as a colourless oil. ^1H NMR (600 MHz) (mixture): 5.487 (α) (1H, br d, $J = 4.3$ Hz), 5.436 (β) (2.6H, br s), 4.634-4.527(α/β) (3.6H, m), 3.977(β) (2.6H, m), 3.846(α) (1H, m), 0.974(β) (7.8H, s), 0.947(α) (3H, d, $J = 6.4$ Hz), 0.943(β) (7.8H, d, $J = 6.6$ Hz), 0.897(α) (3H, s), 0.872(β) (7.8H, s), 0.861(β) (7.8H, d, $J = 6.8$ Hz), 0.790(β) (7.8H, d, $J = 6.8$ Hz).

3-Benzoyloxy-cholest-4-ene (21). ^1H NMR (600 MHz) (**21a**): 5.455 (1H, m), 4.594 (1H, d, $J = 12.2$ Hz), 4.519 (1H, d, $J = 12.2$ Hz), 3.771 (1H, m), 2.205 (1H, m), 0.977 (3H, s), 0.904 (3H, d, $J = 6.6$ Hz), 0.867 (3H, d, $J = 6.6$ Hz), 0.863 (3H, d, $J = 6.6$ Hz), 0.680 (1H, s). ^{13}C NMR (151 MHz): 150.6, 139.4, 128.3 (2 C), 127.7 (2 C), 127.3, 118.7, 70.9, 70.0, 56.2, 56.1, 53.5, 42.5, 39.9, 39.5, 37.6, 36.2, 35.9, 35.8, 32.7, 32.5, 32.1, 28.2, 28.1, 24.3, 24.2, 23.8, 22.8, 22.6, 21.5, 18.7, 18.2, 12.0. ^1H NMR (600 MHz) (**21b**): 5.389 (1H, m), 4.601 (1H, d, $J = 11.9$ Hz), 4.561 (1H, d, $J = 11.9$ Hz), 3.944 (1H, m), 2.200 (1H, m), 1.813 (1H, m), 1.052 (3H, s), 0.901 (3H, d, $J = 6.6$ Hz), 0.866 (3H, d, $J = 6.6$ Hz), 0.861 (3H, d, $J = 6.6$ Hz), 0.677 (1H, s). ^{13}C NMR (151 MHz): 148.0, 139.1, 128.3 (2 C), 127.7 (2 C), 127.3, 120.9, 74.7, 69.8, 56.2 (2 C), 54.5, 42.5, 39.9, 39.5, 37.6, 36.2, 36.0, 35.8, 35.4, 33.1, 32.3, 28.2, 28.0, 25.8, 24.2, 23.8, 22.8, 22.5, 21.0., 18.8, 18.7, 12.0.

3-Benzoyloxy-ergost-8(14)-en-3,4-diol (14). A solution of **13** (165.3 mg, 0.3 mmol) in 4 mL of anhydrous THF was stirred at 0 °C under a N_2 atmosphere for 10 min before adding 0.6 mL of 2 M borane-dimethyl sulfide slowly via a gas-tight syringe. After 15 min, the reaction was removed from the ice bath and stirred at room temperature for 4 h before adding 1 mL of 6 M NaOH dropwise. After 15 min, 1 mL of 30% H_2O_2 was added dropwise and the reaction mixture was stirred vigorously at 50 °C for 16 h. The reaction was allowed to cool to room temperature, quenched with water and extracted with 4:1 hexanes/EtOAc. The organic layer was washed with brine and sodium bicarbonate, dried over Na_2SO_4 and evaporated under reduced pressure. Silica gel chromatography with 19:1 hexanes/ EtOAc yielded

81.6 mg of an inseparable 2.6:1 (3 β ,4 α ,5 α /3 α ,4 β ,5 β) of **14** (48% yield). ¹H NMR (600 MHz) (mixture): 4.715 (3 α) (1H, d, *J* = 11.5 Hz), 4.711 (3 β) (1H, d, *J* = 11.6 Hz), 4.496 (3 α) (1H, d, *J* = 11.5 Hz), 4.487(3 β) (1H, d, *J* = 11.7 Hz), , 3.970(3 α) (1H, dd, *J* = 8.9, 10.8 Hz), 3.417(3 β) (1H, dd, *J* = 8.8, 10.3 Hz), 3.293-3.193(3 α /3 β) (2H, m), 2.433(3 β) (1H, ddd, *J* = 2.1, 4.5, 14.1 Hz), 0.932(3 β) (3H, d, *J* = 6.6 Hz), 0.857(3 α) (3H, d, *J* = 7.0 Hz), 0.855(3 β) (3H, d, *J* = 7.0 Hz), 0.835(3 β) (3H, s), 0.829 (3 α) (3H, s), 0.784 (3 β) (3H, d, *J* = 6.8 Hz), 0.782 (3 β) (3H, d, *J* = 6.8), 0.732 (3 β) (3H, s).

3-Benzoyloxy-cholestan-3,4-diol (22). ¹H NMR (600 MHz) (mixture): 4.703 (3 α ,5 β) (1H, d, *J* = 11.5 Hz), 4.699 (3 β ,5 α) (1H, d, *J* = 11.6 Hz), 4.476 (3 α ,5 β) (1H, d, *J* = 11.5 Hz), 4.476 (3 β ,5 α) (1H, d, *J* = 11.6 Hz), 3.864 (3 α ,5 β) (1H, m), 3.415 (3 β ,5 α) (1H, m), 3.228 (3 α ,5 β) (1H, m), 3.180 (3 β ,5 α) (1H, m), 2.519 (3 α ,5 β) (1H, d, *J* = 1.4 Hz), 2.481 (3 β ,5 α) (1H, d, *J* = 1.4 Hz), 0.898 (3 β ,5 α) (3H, d, *J* = 6.6 Hz), 0.866 (3 β ,5 α) (3H, d, *J* = 6.6 Hz), 0.861 (3 β ,5 α) (3H, d, *J* = 6.6 Hz), 0.840 (3 β ,5 α) (3H, s), 0.648 (3 β ,5 α) (3H, s).

3-Benzoyloxy-ergost-8(14)-en-4-one (15). To a solution of **14** (81.6 mg, 0.16 mmol) in 5 mL of CH₂Cl₂ was added PCC (132.6 mg, 0.61 mmol). The mixture was stirred for 1 h. Hexanes (5 mL) was added to the mixture before filtering it through silica gel. The crude product was eluted with 4:1 hexanes/EtOAc to yield a 2.6:1 (3 β ,5 α /3 α ,5 β) mixture of **15** as a white solid (71.2 mg, 95% yield). **(3 α ,5R)-3-Benzoyloxy-ergost-8(14)-en-4-one (15a)** ¹H NMR (600 MHz): 4.872 (1H, d, *J* = 11.8 Hz), 4.500 (1H, d, *J* = 11.9 Hz), 3.892 (1H, m), 0.953(3H, s), 0.917 (3H, d, *J* = 6.7 Hz), 0.855 (3H, d, *J* = 6.8 Hz), 0.814 (3H, s), 0.784 (3H, d, *J* = 6.8 Hz), 0.780 (3H, d, *J* = 6.8 Hz). **(3 β ,5S)-3-Benzoyloxy-ergost-8(14)-en-4-one (15b)** ¹H NMR (600 MHz): 4.852 (1H, d, *J* = 11.9 Hz), 4.475 (1H, d, *J* = 12.0 Hz), 3.971 (1H, m), 2.469 (1H, ddd, *J* = 1.4, 5.0, 13.6 Hz), 0.932 (3H, d, *J* = 6.6 Hz), 0.855 (3H, d, *J* = 6.8 Hz), 0.826 (3H, s), 0.785 (3H, d, *J* = 6.8 Hz), 0.782 (3H, d, *J* = 6.8 Hz), 0.642, (3H, s).

3-Benzyloxy-cholestan-4-one (23). ^1H NMR (600 MHz) (mixture): 4.861 (1H, d, $J = 12.1$ Hz), 4.846 (1H, d, $J = 12.0$ Hz), 4.490 (1H, d, $J = 12.0$ Hz), 4.464 (1H, d, $J = 12.0$ Hz), 3.937 (1H, dd, $J = 7.4, 12.0$ Hz), 3.828 (1H, dd, $J = 6.6, 12.3$ Hz), 0.899 (3H, d, $J = 6.5$ Hz), 0.866 (3H, d, $J = 6.6$ Hz), 0.862 (3H, d, $J = 6.6$ Hz), 0.747 (3H, s), 0.648 (3H, s), 0.626 (3H, s).

(3 β ,5S)-3-Benzyloxy-cholestan-4-one (23b). A solution of 3-benzyloxy-cholestan-4-one **20** (585.3 mg, 1.2 mmol) in 10 mL of 10% methanolic NaOH was heated at reflux for 1 h. The solvent was removed under reduced pressure before extraction of the product with 5% hydrochloric acid and 2:1 hexane/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to afford **21** as a white solid (580.8 mg, 99% yield). ^1H NMR (600 MHz): 4.845 (1H, d, $J = 12.0$ Hz), 4.464 (1H, d, $J = 12.0$ Hz), 3.936 (1H, dd, $J = 7.3, 12.1$ Hz), 2.252 (1H, m), 2.079 (1H, dd, $J = 2.8, 12.1$ Hz), 0.898 (3H, d, $J = 6.5$ Hz), 0.866 (3H, d, $J = 6.6$ Hz), 0.861 (3H, d, $J = 6.6$ Hz), 0.746 (3H, s), 0.647 (3H, s). ^{13}C NMR (151 MHz): 210.5, 138.2, 128.4 (2 C), 127.8 (2 C), 127.6, 81.3, 71.8, 57.5, 56.3, 56.2, 54.5, 43.0, 42.6, 39.9, 39.5, 36.4, 36.1, 35.7, 34.9, 30.4, 28.2, 28.0, 24.1, 23.8, 22.8, 22.5, 21.7, 20.3, 18.7, 13.6, 12.0.

3 β -Benzyloxy-ergost-8(14)-en-4-methylene (16). A solution of methyltriphenylphosphonium bromide (103.6 mg, 0.26 mmol) and potassium tert-butoxide (28.4 mg, 0.24 mmol) in 500 μL of anhydrous THF was heated at reflux under a N_2 atmosphere for 30 min. A solution of **15** (20.2 mg, 0.04 mmol) in 1 mL of anhydrous THF was transferred to the generated ylide via a gas-tight syringe. The reaction was refluxed under a N_2 atmosphere for an additional 14 h. The reaction was cooled to rt, quenched with water and extracted with 4:1 hexane/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. Silica chromatography with 39:1 hexanes/EtOAc yielded a mixture of 4 products **14** (18.8 mg, 93% yield): desired product **14c** 79%, 6% **14a**, 7% **14b** and 8% **14d**. The desired product was isolated by preparative TLC with 39:1 hexanes/EtOAc. **(5S)-3 β -Benzyloxy-ergost-8(14)-en-4-methylene (16c)** ^1H NMR (600 MHz): 5.214 (1H, br d, $J = 1.3$ Hz), 4.699 (1H, d, $J = 12.2$

Hz), 4.647 (1H, br d, $J = 1.4$ Hz), 4.566 (1H, d, $J = 12.1$ Hz), 3.719 (1H, dd, $J = 5.0, 11.7$ Hz), 2.475 (1H, ddd, $J = 2.2, 4.1, 13.9$ Hz), 1.938 (1H, dt, $J = 3.4, 12.4$ Hz), 0.937 (3H, d, $J = 6.6$ Hz), 0.858 (3H, d, $J = 6.9$ Hz), 0.835 (3H, s), 0.788 (3H, d, $J = 6.8$ Hz), 0.786 (3H, d, $J = 6.8$ Hz), 0.607, (3H, s).

3 β -Benzyloxy-4-methylenecholestane (25). ^1H NMR (600 MHz): 5.191 (1H, br d, $J = 1.2$ Hz), 4.697 (1H, d, $J = 12.1$ Hz), 4.651 (1H, br d, $J = 1.3$ Hz), 4.558 (1H, d, $J = 12.2$ Hz), 3.689 (1H, dd, $J = 5.2, 11.7$ Hz), 0.909 (3H, , d, $J = 6.5$ Hz), 0.874 (3H, d, $J = 6.6$ Hz), 0.870 (3H, d, $J = 6.6$ Hz), 0.708 (3H, d, $J = 6.6$ Hz), 0.708, (3H, s), 0.659 (3H, s). ^{13}C NMR (151 MHz): 1502.1, 139.2, 128.3 (2 C), 127.3 (3 C), 103.4, 80.6, 71.1, 56.34, 56.33, 54.6, 50.3, 42.6, 40.1, 39.5, 38.9, 37.5, 36.2, 35.8, 35.3, 31.7, 30.6, 28.3, 28.0, 24.3, 24.2, 23.8, 22.8, 22.6, 21.8, 18.7, 12.9, 12.1.

4-Methylene-ergost-8(14)-en-3-ol (17). To a solution of 0.5 cm of Li wire in 2.5 mL of condensed NH_3 at -78°C (acetone/dry ice bath) was added **16** (10.2 mg, 0.02 mmol) in 2.5 mL of Et_2O . The reaction was stirred at -78°C for 10 min and quenched with 1 mL of MeOH. The reaction was warmed to rt, diluted in 10% hydrochloric acid and extracted with 2:1 hexane/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under a flow of N_2 . The crude products were purified by preparative TLC to obtain a mixture of **17** as a solid (8.2 mg, 98% yield). The 4 alcohols were separated by high-performance liquid chromatography with MeOH. **(3 α ,5R)-4-Methylene-ergost-8(14)-en-3-ol (17a).** ^1H NMR (600 MHz): 4.968 (1H, m), 4.604 (1H, m), 4.294 (1H, m), 0.941 (C21) (3H, d, $J = 6.6$ Hz), 0.858 (C26/27) (3H, d, $J = 6.8$ Hz), 0.839 (C18) (3H, s), 0.788(C26/27) (3H, d, $J = 6.9$ Hz), 0.785 (C28) (3H, d, $J = 6.8$ Hz), 0.580 (C19) (3H, s). **(3 α ,5S)-4-Methylene-ergost-8(14)-en-3-ol (17b).** ^1H NMR (600 MHz): 5.296 (1H, br s), 4.987 (1H, br s), 4.006 (1H, m), 0.925 (C21) (3H, d, $J = 6.6$ Hz), 0.880 (C19) (3H, s), 0.855 (C26/27) (3H, d, $J = 6.8$ Hz), 0.827 (C18) (3H, s), 0.784 (C26/27) (3H, d, $J = 6.8$ Hz), 0.782 (C28) (3H, d, $J = 6.8$ Hz). **(3 β ,5R)-4-Methylene-ergost-8(14)-en-3-ol (17c or 3).** ^1H NMR (600 MHz): 5.076 (1H, s), 4.633 (1H, s), 4.023 (1H, m), 0.939 (C21) (3H, d, $J = 6.6$ Hz), 0.853 (C26/27) (3H, d, $J = 6.8$ Hz), 0.838 (C18) (3H, s), 0.786 (C26/27) (3H, d, $J = 6.9$ Hz), 0.786(C28) (3H, d, $J = 6.8$ Hz),

0.592 (C19) (3H, s). **(3 β ,5S)-8(14)-4-Methylene-ergost-8(14)-en-3-ol (17d)** ^1H NMR (600 MHz): 5.154 (1H, m), 4.938 (1H, m), 4.304 (1H, m), 2.435 (1H, dd, $J = 2.3, 4.6$ Hz) 0.928(C21) (3H, d, $J = 6.7$ Hz), 0.911 (C19) (3H, s), 0.854 (C26/27) (3H, d, $J = 6.9$ Hz), 0.833 (C18) (3H, s), 0.783 (C26/27) (3H, d, $J = 6.8$ Hz), 0.782 (C28) (3H, d, $J = 6.8$ Hz).

24-epitheonellasterol (4): ^1H NMR (600 MHz): 5.075 (1H, br s), 4.632 (1H, br s), 4.019 (1H, m), 2.478 (1H, ddd, $J = 2.4, 4.4, 13.9$ Hz), 0.941 (C21) (3H, d, $J = 6.6$ Hz), 0.851 (C29) (3H, d, $J = 7.4$ Hz), 0.840 (C18) (3H, s), 0.840 (C26/27) (3H, d, $J = 6.8$ Hz), 0.818 (C26/27) (3H, d, $J = 6.9$ Hz), 0.591 (C19) (3H, s). ^{13}C NMR (151 MHz): 153.2, 143.0, 125.7, 102.8, 73.4, 56.8, 49.5, 49.3, 46.0, 42.8, 40.0, 37.4, 36.8, 34.8, 33.8, 33.2, 29.4, 29.2, 27.1, 26.0, 25.8, 24.7, 23.1, 20.4, 19.8, 19.2, 19.1, 18.2, 13.2, 12.0.

24-desmethylconicasterol: ^1H NMR (600 MHz): 5.075 (1H, m), 4.633 (1H, br s), 0.934 (C21) (3H, d, $J = 6.7$ Hz), 0.870 (C26/C27) (3H, d, $J = 6.6$ Hz), 0.866 (C26/C27) (3H, d, $J = 6.6$ Hz), 0.839 (C18) (1H, s), 0.591 (C19) (3H, s).

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CHAPTER 3

Synthesis of Pfiesterol and its 23-Epimer from 16-Dehydropregnenolone Acetate

Abstract

Pfiesterol, (23R)-4 α ,23-dimethylergost-17(20),24(28)-dien-3 β -ol, a sterol from the heterotrophic dinoflagellate *Pfiesteria picicida*, was synthesized from 16-dehydropregnenolone acetate. This compound is a potential biomarker for the species *Pfiesteria*. Two different routes to the pfiesterol from intermediate **18b**, (23R)-23-methylergost-4,17(20),24(28)-trien-3-one, were used differing in the approach to stereoselectively monomethylate at C4. The 4 α -(¹³C-methyl) pfiesterol was also synthesized via a reductive methylation with labeled methyl iodide. The labeled product was prepared for metabolic studies in copepods. Pfiesterol is also potential precursor of 4-methylandrosterone, an androgenic sex hormone that may affect invertebrate reproduction.

3.1 Introduction

Many sterols have been used as biomarkers for marine and terrestrial organisms including dinoflagellates.¹⁻³ Dinoflagellates are an important and diverse group of motile marine and fresh water phytoplankton with the ability to produce potent toxins harmful to fish, shellfish and human health.^{4,5,8c,11}

Dinosterol, 4 α -methyl sterols, 23-methylsterols, and some steroid ketones are considered biomarkers for dinoflagellates.^{3,6-7} Brevesterol, an unusual sterol from the harmful dinoflagellate *K. brevis*, has been proposed as biomarker for the species.⁹ A new sterol isolated from two strains of the toxic dinoflagellate *Pfiesteria piscicida*¹², pfiesterol ((23R)-4 α ,23-dimethylergost-17(20),24(28)-dien-3 β -ol) (**1**), was identified as a potential biomarker for that species.^{8c} A published analysis of the sterols in *Pfiesteria piscicida* did not report that compound even though not all the isolated sterols were identified.¹¹ The unusual $\Delta^{17(20)}$ functionality in pfiesterol has been previously observed in the dinoflagellate sterol peridinosterol ((23R,24R)-4 α ,23,24-trimethyl-cholest-17(20)-en-3 β -ol).^{8b,13} The stereochemistry assignments in the nucleus and side chain of pfiesterol were achieved by chemical correlation through heterogenous catalytic hydrogenation of pfiesterol to give 20-epidihydrodinosterol, (20S,23R,24R)-4 α ,23-trimethyl-cholestan-3 β -ol, as the major product.^{13a} The side chain of pfiesterol is not terribly stable. A sample of pfiesterol stored in a freezer in CDCl₃ decomposed to give 4 α -methylandrosterone, an androgenic steroid hormone.^{8c} In vivo and in vitro experiments demonstrated that vertebrate related steroids like androgens directly and indirectly affect reproduction in marine molluscs through biological effects that include induction of sex reversal.¹⁴ Two methods of synthesizing pfiesterol from the commercially available steroid 16-dehydropregnenolone acetate, differing in the introduction of the 4 α -methyl group, were established. The 23-epimer of pfiesterol (**3**) and 4 α -(¹³C-methyl) pfiesterol (**1***) were also synthesized and characterized by NMR (Figure 3.1). The label in compound **1*** was strategically added to the nucleus to allow an easy and unambiguous identification of the side chain decomposition product, 4 α -¹³C-methyl androsterone.

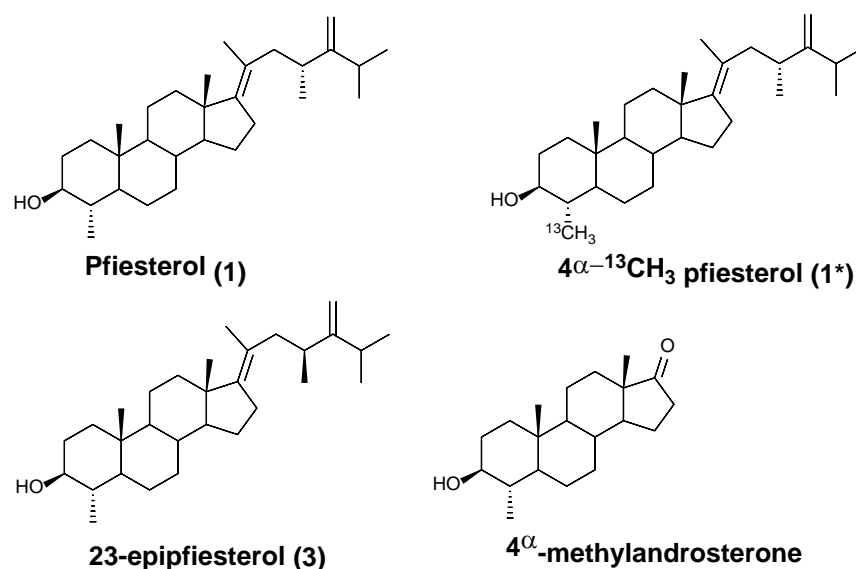
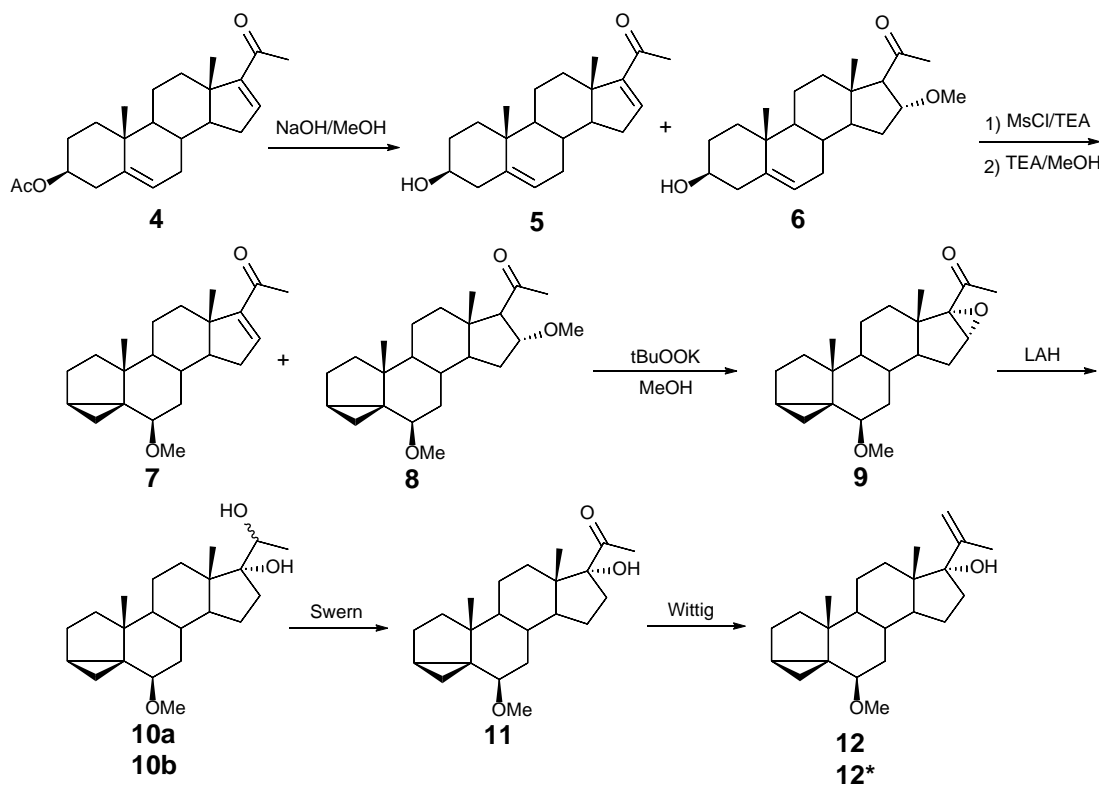


Figure 3.1. Pfisterol (**1**), its 23-epimer (**3**), 4α-¹³C-methyl-pfisterol (**1***) and 4α-methylandrosterone.

3.2 Results and Discussion

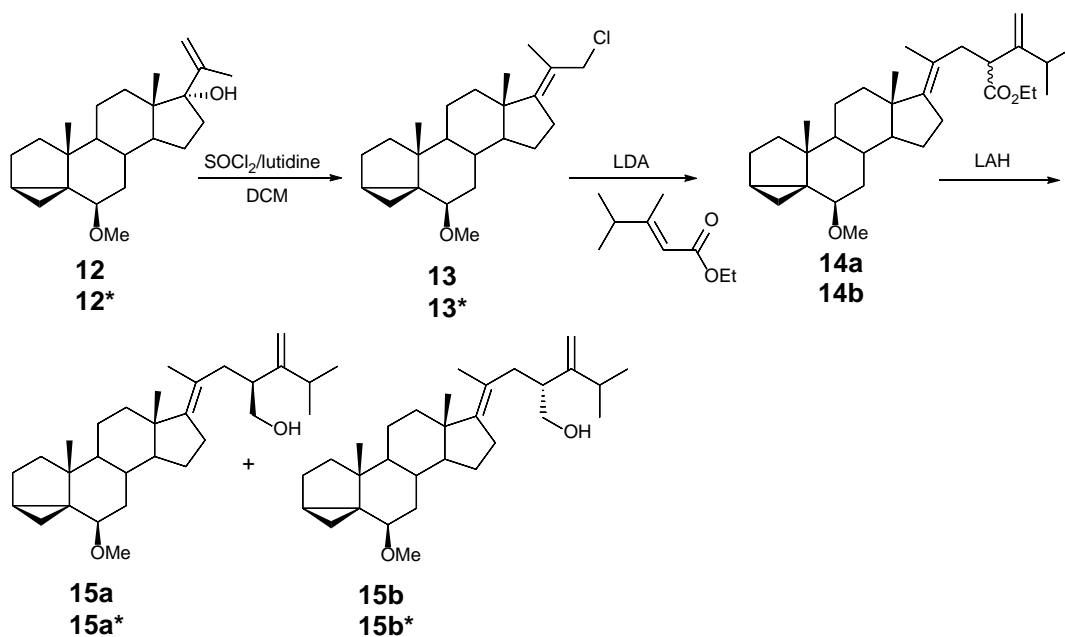
The synthesis started with the saponification of 16-dehydropregnenolone acetate (**4**) to obtain 16-dehydropregnenolone **5**¹⁵⁻¹⁶ and the Michael-addition product **6**¹⁷ in a 3:2 mixture favoring compound **5** (Scheme 3.1). The use of tBuOH, a more hindered alcohol, has been reported not to give the Michael-addition product¹⁵. We decided to move forward with the mixture because the formation of more Michael-addition products was expected in the following step due the methanolic NaOH treatment involved in the i-methyl ether formation. The alcohol mixture was subsequently protected to its respective i-methyl sterols in a two-step procedure to afford a 2:3 mixture of compounds **7**^{16,18} and **8**. The mixture of i-methyl sterols was converted in high yield to the epoxide **9** upon treatment with methanolic tBuOOK at room temperature.¹⁹ This reaction successfully converted the starting mixture into one product, the epoxide **9**, by regenerating the Δ¹⁶ in the Michael-addition product via a retro-Michael addition of the methoxy group before its subsequent epoxidation. The keto-epoxide **9** was reduced with LAH to afford a mixture

of diols **10a** and **10b** followed by a Swern oxidation²⁰ to obtain the keto-alcohol **11**²¹ in an 85% yield. A better yield was obtained with Dess-Martin Periodinane (DMP), however, that reaction would be too expensive to do on a large scale. Oxidation with PCC mainly gave the oxidative cleavage product, 6 β -methoxy-3 α ,5 α -cycloandrostan-17-one²². The keto-alcohol **11** was converted to the tertiary allylic alcohol **12** via a Wittig reaction. Some minor side products interpreted as the methylenated acyloin rearrangement²³ products were observed from that reaction. Introduction of ¹³C-label via the Wittig reaction gave compound **12***, an intermediate in a sequence to (23R)-(22-¹³C23)-methylelgergost-17(20),24(28)-dien-3 β -ol (**17b***).



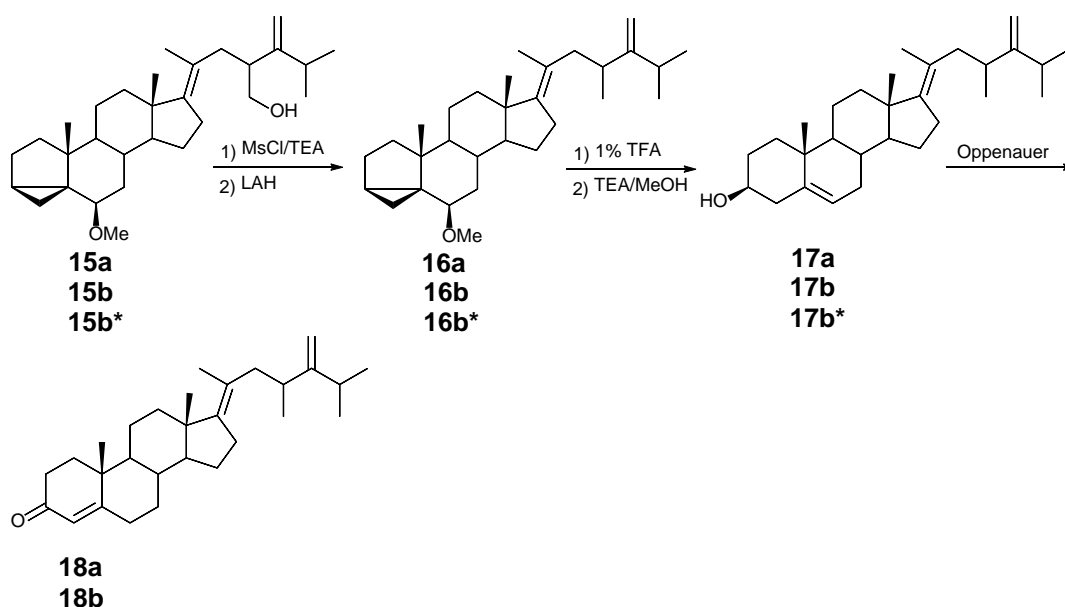
Scheme 3.1. Synthesis of intermediate **12** from 16-dehydroprenenolone.

As shown in Scheme 3.2, the allylic alcohol **12** was converted to the desired E-allylic chloride **13** as the major product with thionyl chloride and lutidine in DCM.²³ The product was not further purified due to its observed instability to exposure to silica gel and alumina. The crude product was stable when stored in benzene at -20 °C. That reaction was important in this sequence because it provided the $\Delta^{17(20)}$ functionality in the desired product and activated the substrate for a facile SN2 reaction in the next step. The remaining carbons in the side chain of the target compound were added via the alkylation of the lithium enolate of (E)-3,4-dimethyl-2-pentenoate with the i-methyl ether chloride **13** at -78 °C.²⁴ The resulting esters **14a** and **14b** were poorly separated on silica gel, thus, they were reduced with LAH to get a mixture of the alcohols **15a** and **15b** that were easier to separate by chromatography. The two alcohols were isolated and carried separately through the rest of the sequence. The separation of alcohols **15b** and **15a** allowed a direct synthesis of pfiesterol and 23-epipfiesterol, respectively.



Scheme 3.2. Synthesis of the alcohols **15a** and **15b** from compound **12**.

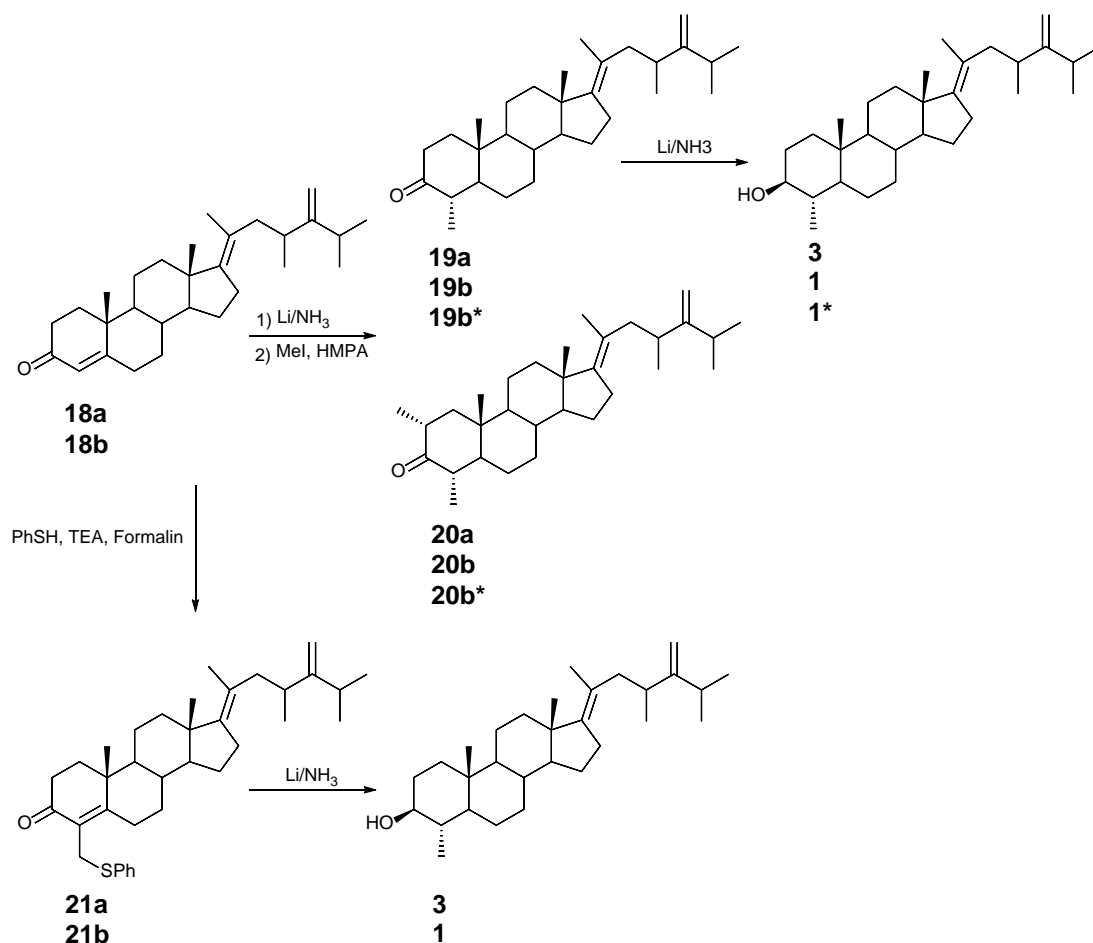
The alcohol i-methyl ethers **15a** and **15b** were deoxygenated via the reduction of their respective mesylates to afford compounds **16a** and **16b** (Scheme 3.3). Minor starting alcohols were recovered from the LAH reduction of the mesylates from undesired S-O cleavage. With the desired side chains in place, the i-methyl ethers **16a** (23S) and **16b** (23R) were deprotected to obtain their corresponding sterols. The sterols **17a** and **17b** were converted to the enones **18a** and **18b** in good yield using the Oppenauer oxidation²⁵.



Scheme 3.3. Synthesis of the enones **18a** and **18b** from the alcohols **15a** and **15b**.

The enones were used in two different methods to generate pfiesterol and 23-epipfiesterol (Scheme 3.4). The first method to pfiesterol and its 23-epimer from their corresponding enone involved the Kirk-Petrow reaction.²⁶ This method was previously in our lab to make pfiesterol from the Δ^5 isomer of the enone **18b**.^{9a} The phenylthiomethyl intermediates **21a** and **21b** were easily converted to 23-epipfiesterol and pfiesterol via a dissolving metal reduction (Li/NH_3), respectively.^{9a} The second method

involved a reductive methylation.²⁷ The reductive methylation of both enones gave a mixture of their respective desired monomethylated ketone (**19a/19b**) and the dimethylated product (**20a/20b**) in a 2:1 ratio favoring the monomethylated product. The unsaturated ketone and the fully reduced starting material were observed as minor side products. That reaction was almost impossible to run predictably and successfully in the summer due to the high sensitivity of the enolate to humidity. Dry solvents and reagents and oven dry glassware were crucial in getting the reaction to work. However, the reaction still did not work at high humidity. The reductive alkylation was not that selective, however, it allowed a relatively cheap and easy method to introduce the ¹³C-methyl in **1*** at a late stage in the synthesis. The labeled product (**19b***) was generated using this method with ¹³CH₃I. Pfiesterol (**1**), 23-epipfiesterol (**3**), and 4 α -(¹³C-methyl) pfiesterol (**1***) were generated as the exclusive products from **19a**, **19b** and **19b*** via a reductive metal reduction (Li/NH₃), respectively.²⁷ Hydride reduction of the 4 α -methyl ketone gives 21% of the undesired 3 α -hydroxy product.



Scheme 3.4. Synthesis of 23-epipfiesterol **3** and pfiesterol **1** from the enones **18a** and **18b**.

3.3 Summary

Pfiesterol and its 23-epimer were successfully synthesized from 16-dehydroprenolone acetate. Two different methods were used to generate the 4α -methyl nucleus from the Δ^4 -enone intermediates **18a** and **18b**. The structure of the synthesized pfiesterol was confirmed by ^1H NMR comparison with the natural product and a sample previously prepared in our lab.^{8b} The labeled product was synthesized via a reductive methylation of intermediate **18b** with $^{13}\text{CH}_3\text{I}$. Introducing the label this late in the synthesis

provided an economic method compound 1*. The synthesized 4 α -(¹³C-methyl) pfiesterol provided samples for metabolic studies to verify the in vivo formation of the hormone 4 α -¹³C-methylandrosterone in invertebrates from the side chain degradation of 4 α -(¹³C-methyl) pfiesterol. This steroid hormone may negatively affect reproduction in invertebrates. A sample of pfiesterol stored in the freezer in benzene for over 2.5 years was found unchanged by ¹H NMR analysis.

3.4 Experimental

General Procedures. NMR spectra were obtained in CDCl₃ on a Bruker 600 MHz spectrometer (¹H at 600 MHz and ¹³C at 151 MHz). HPLC was performed using a Waters 6000A pump, Waters 410 differential refractometer, and two Altex Ultrasphere ODS columns (5 μ m, 10 x 250 mm) in series at a flow rate of 3 mL/min with MeOH. Preparative thin layer chromatography (TLC) purifications were done with Sorbent Technologies (SORBTECH) silica gel HL thin TLC plates (20 x 20 cm², 250 μ m thick with glass backed support). All solvents were from commercial sources.

16-Dehydropregnenolone (5) and 16- α -methoxypregnenolone (6). To a solution of 4 (15.1 g, 42.4 mmol) in 50 mL of 4:1 MeOH/THF was added 20 mL of 10% methanolic NaOH. The mixture was heated at reflux for 1 h. The reaction was cooled to rt before removing the solvent under reduced pressure. The crude product was extracted with saturated sodium bicarbonate and 2:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and filtered through silica gel before removing the solvent under reduced pressure. A 3:2 mixture of compound **5** and **6**, respectively, was obtained as a white solid (12.13g, 88% yield). **16-Dehydropregnenolone (5).** ¹H NMR (600 MHz): 6.703 (1H, dd, *J* = 1.85, 3.16 Hz), 5.362 (1H, m), 3.525 (1H, m), 2.260 (3H, s), 1.049 (3H, s), 0.923 (3H, s). ¹³C NMR (151 MHz): 196.8, 155.4, 144.4, 141.4, 121.1, 71.7, 56.5, 50.5, 46.1, 42.3, 37.1, 36.7, 34.7, 32.3, 31.7, 31.6, 30.2, 27.1, 20.7, 19.3, 15.7. **16 α -Methoxypregnenolone (6).** ¹H NMR (600 MHz): 5.353 (1H, m), 4.355 (1H,

m), 3.535 (1H, m), 3.217 (3H, s), 2.179 (3H, s), 1.005 (3H, s), 0.639 (3H, s). ^{13}C NMR (151 MHz): 208.1, 140.7, 121.3, 81.5, 71.7, 71.6, 57.2, 54.5, 49.9, 44.4, 42.2, 38.8, 37.1, 36.5, 32.0, 31.7, 31.6 (2 C), 31.5, 20.8, 19.4, 14.5.

6 β -Methoxy-3 α ,5-cyclo-pregn-16-en-3-one (7) and 6 β ,16 α -Dimethoxy-3 α ,5 α -cyclo-pregnan-20-one (8). To a solution of 13.12 g of a 3:2 mixture of compounds **5** (23.1 mmol) and **6** (14.0 mmol) in 150 mL of dry DCM was added 12 mL of TEA. The mixture was stirred at 0 °C for 10 min before adding 9 mL of 1:1 DCM/MsCl dropwise over 15 min. The mixture was stirred for an additional 45 min before extracting the reaction with saturated sodium bicarbonate and 9:1 hexanes/EtOAc. The organic layer was with brine, dried over Na_2SO_4 and filtered through silica gel before removing the solvent under reduced pressure. The crude mixture of the mesylates (14.9 g) was redissolved in 200 mL of 3:1 MeOH/DCM and 25 mL of 10% methanolic NaOH and stirred at reflux for 2 h. The reaction was cooled to rt before extraction with 10% HCl and 4:1 hexanes/EtOAc. The combined organic layers were washed with saturated sodium bicarbonate, then brine, and dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash silica gel chromatography with 9:1 hexanes/EtOAc to afford of a 2:3 mixture of compounds **7** and **8**, respectively (12.81 g, 84% yield for the two steps). **6 β -Methoxy-3 α ,5 α -cyclo-pregn-16-en-3-one (7).** ^1H NMR (600 MHz): 6.691 (1H, dd, $J = 1.85, 3.30$ Hz), 3.355 (3H, s), 2.804 (1H, t, $J = 2.8$ Hz), 2.254 (3H, s), 1.057 (3H, s), 0.947 (3H, s), 0.677 (1H, dd, $J = 4.1, 4.8$), 0.456 (1H, dd, $J = 5.2, 8.0$ Hz). ^{13}C NMR (151 MHz): 196.8, 155.6, 144.3, 82.2, 56.7, 56.5, 48.5, 46.4, 43.6, 35.4, 35.2, 35.1, 33.1, 32.2, 28.9, 27.1, 24.9, 22.3, 21.3, 19.2, 16.2, 13.2. **6 β ,16 α -Dimethoxy-3 α ,5 α -cyclo-pregnan-20-one (8).** ^1H NMR (600 MHz): 4.347 (1H, m), 3.323 (3H, s), 3.208 (3H, s), 2.779 (1H, t, $J = 2.8$ Hz), 2.543 (1H, d, $J = 6.2$ Hz), 2.168 (3H, s), 1.016 (3H, s), 0.668 (3H, s), 0.451 (1H, dd, $J = 5.1, 8.0$ Hz). ^{13}C NMR (151 MHz): 208.1, 82.1, 81.5, 71.8, 57.1, 56.6, 54.3, 47.9, 44.9, 43.4, 39.4, 35.1, 35.0, 33.3, 31.9, 31.7, 30.1, 24.9, 22.4, 21.4, 19.2, 14.9, 13.1.

(16R,17R)-6 β -Methoxy-3 α ,5 α -cyclo-16,17-epoxy-pregnan-20-one (9). To a solution of 13.5 g of a mixture of compounds **7** (16.4 mmol) and **8** (22.4 mmol) in 50 mL of 4:1 MeOH/THF and 6.5 mL of 70% tBuOOH in foil-wrapped flask was added 46.8 mL of 6 M KOH over 20 min. Reaction was checked by NMR until no starting material was observed. After 42 h, the reaction was extracted with water and 2:1 hexanes/EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄ and filtered through a small pad of silica gel. The crude product was isolated as a white solid after removing the solvent under reduced pressure (13.03 g, 96% pure by NMR). ¹H NMR (600 MHz): 3.669 (1H, s), 3.331 (3H, s), 2.778 (1H, t, *J* = 2.8 Hz), 2.028 (3H, s), 1.081 (3H, s), 1.034 (3H, s), 0.667 (1H, m), 0.447 (1H, dd, *J* = 5.2, 8.0 Hz). ¹³C NMR (151 MHz): 204.9, 82.1, 71.1, 60.6, 56.7, 48.4, 45.4, 43.6, 42.0, 35.1, 35.0, 33.2, 31.8, 28.2, 27.4, 26.0, 24.9, 22.0, 21.2, 19.2, 15.6, 13.2.

(17R)-6 β -Methoxy-3 α ,5 α -cyclo-pregnan-17,20-diol (10). To a solution of the epoxy-ketone **9** (13.0 g, 37.7 mmol) in 100 mL of anhydrous Et₂O was added 2.86 g of LAH dissolved in 25 mL of Et₂O via an addition funnel over 10 min. The reaction was stirred for 2 h before extraction with 10% hydrochloric acid and 2:1 hexanes/EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄ and filtered through a small pad of silica gel. The crude product of 2.8:1 of **10a/10b** was isolated as a white solid after removing the solvent mixture under reduced pressure (13.02 g, 99% crude yield). A sample of the mixture was separated by preparative TLC with 4:1 hexanes/EtOAc to provide the compounds **10a** (major and less polar product) and **10b**. **(17R)-6 β -Methoxy-3 α ,5 α -cyclo-pregnan-17,20-diol (10a).** ¹H NMR (600 MHz): 4.040 (1H, dd, *J* = 6.2, 12.5 Hz), 3.321 (3H, s), 2.766 (1H, t, *J* = 2.7 Hz), 1.167 (3H, d, *J* = 6.3 Hz), 1.026 (3H, s), 0.850 (3H, s), 0.645 (1H, m), 0.430 (1H, dd, *J* = 5.2, 7.9 Hz). ¹³C NMR (151 MHz): 85.2, 82.3, 70.4, 56.6, 50.2, 47.7, 47.5, 43.4, 35.2, 35.17, 33.9, 33.4, 32.8, 30.6, 24.9, 23.7, 22.4, 21.4, 19.3, 18.6, 15.5, 13.1. **(17R)-6 β -Methoxy-3 α ,5 α -cyclo-pregnan-17,20-diol (10b).** ¹H NMR (600 MHz): 3.857 (1H, m), 3.332 (3H, s), 2.781 (1H, t, *J* = 2.7 Hz), 1.192 (3H, d, *J* = 6.4 Hz), 1.030 (3H, s), 0.789 (3H, s), 0.654 (1H, m), 0.443 (1H, dd, *J* = 5.1, 7.9 Hz). ¹³C NMR (151 MHz): 85.6,

82.3, 72.4, 56.6, 51.1, 47.6, 46.1, 43.4, 37.7, 35.2, 35.1, 33.4, 31.6, 30.4, 24.9, 23.3, 22.2, 21.5, 19.2, 18.6, 14.4, 13.1.

(17R)-17-Hydroxy-6 β -methoxy-3 α ,5-cyclo-pregnan-20-one (11). To a solution of oxalyl chloride (3.20 mL, 36.3 mmol) in 60 mL of anhydrous DCM at -78 °C under a N₂ atmosphere was added a mixture of DMSO (3 mL, 41.8 mmol) in 7 mL of DCM dropwise over 10 min. After 15 min, a mixture of the glycols **10a** and **10b** (6.07g, 17.4 mmol) in 10 mL of anhydrous DCM was added dropwise to the reaction. After 1h, TEA (12.2 mL, 87.0 mmol) was added dropwise and the reaction was allowed to warm slowly to room temperature before extraction with saturated sodium bicarbonate and 4:1 hexanes/EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄ and filtered through a silica gel. The product was isolated as an off-white solid after removal of the solvent mixture in vacuo (5.6 g, 92% pure by NMR, 85% overall yield). ¹H NMR (600 MHz): 3.339 (3H, s), 2.786 (1H, t, *J* = 2.8 Hz), 2.775 (1H, s), 2.670 (1H, s), 2.276 (3H, s), 1.032 (1H, s), 0.786 (3H, s), 0.663 (1H, m), 0.453 (1H, dd, *J* = 5.2, 8.0 Hz). ¹³C NMR (151 MHz): 211.8, 90.0, 82.2, 56.6, 50.6, 48.8, 47.6, 43.4, 35.3, 35.2, 33.6, 33.4, 30.5, 30.4, 27.9, 24.9, 24.0, 22.2, 21.4, 19.3, 15.8, 13.2.

(17R)-17-Hydroxy-6 β -methoxy-20-methylene-3 α ,5-cyclo-pregnane (12 and 12*). A solution of Wittig reagent was prepared by heating a mixture of methyltriphenylphosphonium bromide (11.63 g, 32.60 mmol) and of potassium tert-butoxide (3.13 g, 27.9 mmol) in 30 mL of dry THF at reflux for 45 min under a N₂ atmosphere. A solution of keto-alcohol **11** (4.14 g, 11.9 mmol) in 15 mL of dry THF was added via a gas-tight syringe to the generated ylide. The resulting mixture was heated at reflux under a N₂ atmosphere for an additional 1.5 h. The reaction was allowed to cool to room temperature before extraction with water and 4:1 hexanes/EtOAc. The combined organic layers were washed with brine, dried over Na₂SO₄, filtered through a silica gel and concentrated under reduced pressure. The product **12** was isolated as white solid after silica gel chromatography with 39:1-19:1 hexanes/EtOAc (2.93 g, 71% yield). ¹H NMR (600 MHz): 4.994 (1H, br s), 4.918 (1H, m), 3.334 (3H, s), 2.782 (1H, t, *J* = 2.8 Hz),

2.422 (1H, m), 1.840 (3H, br s), 1.031 (3H, s), 0.669 (3H, s), 0.655 (1H, m), 0.441 (1H, dd, $J = 5.2, 8.0$ Hz). ^{13}C NMR (151 MHz): 148.1, 112.6, 86.4, 82.4, 56.6, 50.4, 47.7, 47.4, 43.4, 35.3, 35.2, 35.1, 33.4, 31.5, 31.0, 25.0, 23.1, 22.6, 21.5, 21.1, 19.3, 15.7, 13.1. **(17R)-(22- ^{13}C)-17-Hydroxy-6 β -methoxy-20-methylene-3 α ,5 α -cyclo-pregnane (12*)**. This compound was made from the keto-alcohol **11** and $\text{Ph}_3\text{P}^{13}\text{CH}_2$ as described above. ^1H NMR (600 MHz): 4.992 (1H, d, $J = 155.3$ Hz), 4.917 (1H, d, $J = 155.4$ Hz), 3.335 (3H, s), 2.782 (1H, t, $J = 2.8$ Hz), 2.422 (1H, m), 1.840 (3H, br s), 1.031 (3H, s), 0.669 (3H, s), 0.655 (1H, m), 0.441 (1H, dd, $J = 5.2, 8.0$ Hz). ^{13}C NMR (151 MHz): 112.6 (C22).

(E)-22-Chloride-20-methyl-6 β -methoxy-3 α ,5 α -cyclo-pregn-17(20)-ene (13 and 13*). To solution of the allylic alcohol **12** (1.29 g, 3.80 mmol) and 2,6-lutidine (7.0 mL, 56.3 mmol) in 20 mL of dry DCM at 0 °C under a N_2 atmosphere was added SOCl_2 (940 μL , 13.0 mmol) dropwise. The reaction was stirred for 1 h before extraction with 5% hydrochloric acid and hexanes. The combined organic layers were washed with sodium bicarbonate and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was quickly filtered through alumina with 19:1 hexane/EtOAc to obtain **13** as light-yellow oil (1.32 g, crude). **22-Chloride-20-methyl-6 β -methoxy-3 α ,5 α -cyclo-pregn-17(20)-ene (13)**. ^1H NMR (600 MHz): 4.015 (2H, m), 3.342 (3H, s), 2.797 (1H, t, $J = 2.8$ Hz), 1.815 (3H, m), 1.035 (3H, s), 0.915 (3H, s), 0.665 (1H, m), 0.452 (1H, dd, $J = 5.2, 7.9$ Hz). **(22- ^{13}C)22-Chloride-20-methyl-6 β -methoxy-3 α ,5 α -cyclo-pregn-17(20)-ene (13*)**. ^1H NMR (600 MHz): 4.014 (2H, m, $J_{\text{HC}} = 150.8$ Hz), 3.342 (3H, s), 2.797 (1H, t, $J = 2.8$ Hz), 1.815 (3H, m), 1.035 (3H, s), 0.916 (3H, s), 0.664 (1H, m), 0.451 (1H, dd, $J = 5.3, 7.8$ Hz). ^{13}C NMR (151 MHz): 50.2 (C22).

(23 ξ)-Ethyl-6 β -methoxy-3 α ,5 α -cyclo-23-homoergost-17(20),24(28)-dien-23-oate (14a and 14b). To a solution of diisopropylamine (630 μL , 4.5 mmol) in 5 mL of anhydrous THF at 0 °C under a N_2 atmosphere was added and 1.6 mL of 2.81 M of $n\text{-BuLi}$ in hexane. After 10 min, the mixture was cooled to -78 °C before adding ethyl (E)-3,4-dimethyl-2-pentenoate (800 μL , 4.6 mmol) dropwise. After 1 h, a solution of the crude **13** (1.32 g) in 4 mL of anhydrous THF was added dropwise over 5 min at -78 °C.

HMPA (250 μ L) was then added before allowing the reaction to slowly warm up to rt. After 16 h, the reaction was extracted in 5% hydrochloric acid and 4:1 hexanes/EtOAc. The combined organic layers were washed with sodium bicarbonate and then brine, dried over Na₂SO₄ and concentrated under reduced pressure. The mixture of **14a** and **14b** was isolated as clear oil after silica gel chromatography with 39:1-19:1 hexanes/EtOAc (1.24 g, 69% yield from **12a**). A sample of the mixture was separated by preparative TLC with 19:1 hexanes/EtOAc to isolate pure samples of **14a** and **14b**. **(23S)-Ethyl-6 β -methoxy-3 α ,5 α -cyclo-23-homoergost-17(20),24(28)-dien-23-oate (14a)**. ¹H NMR (600 MHz): 5.057 (1H, s), 4.940 (1H, s), 4.085 (2H, m), 3.335 (3H, s), 3.183 (1H, dd, *J* = 5.5, 9.8 Hz), 2.787 (1H, t, *J* = 2.7 Hz), 1.692 (3H, m), 1.230 (3H, t, *J* = 7.1 Hz), 1.061 (3H, d, *J* = 6.8 Hz), 1.043 (3H, d, *J* = 6.8 Hz), 1.027 (3H, s), 0.885 (3H, s), 0.654 (1H, m), 0.440 (1H, dd, *J* = 5.2, 8.0 Hz). ¹³C NMR (151 MHz): 174.2, 154.0, 146.0, 121.2, 108.9, 82.4, 60.2, 56.6, 56.4, 48.0, 47.9, 44.8, 43.4, 40.0, 38.1, 35.3, 35.0, 34.5, 33.3, 30.0, 29.6, 25.0, 24.3, 23.1, 22.0, 21.5, 19.2, 17.6, 16.5, 14.2, 13.1. **(23R)-Ethyl-6 β -methoxy-3 α ,5 α -cyclo-23-homoergost-17(20),24(28)-dien-23-oate (14b)**. ¹H NMR (600 MHz): 5.051 (1H, s), 4.938 (1H, s), 4.082 (2H, m), 3.334 (3H, s), 3.164 (1H, dd, *J* = 6.5, 8.7 Hz), 2.789 (1H, t, *J* = 2.7 Hz), 1.690 (3H, m), 1.225 (3H, t, *J* = 7.2 Hz), 1.057 (3H, d, *J* = 6.8 Hz), 1.039 (3H, d, *J* = 6.8 Hz), 1.027 (3H, s), 0.868 (3H, s), 0.655 (1H, m), 0.441 (1H, dd, *J* = 5.1, 8.0 Hz). ¹³C NMR (151 MHz): 174.4, 153.9, 146.0, 121.5, 109.0, 82.4, 60.3, 56.6, 56.4, 48.2, 47.9, 44.9, 43.4, 39.8, 37.9, 35.3, 35.0, 34.4, 33.3, 30.0, 29.8, 25.0, 24.3, 23.1, 22.1, 22.0, 21.4, 19.2, 17.5, 16.7, 14.1, 13.1.

(23 ξ)-6 β -Methoxy-3 α ,5 α -cyclo-23-homoergost-17(20),24(28)-dien-23-ol (15a,15b). The esters **14a** and **14b** (1.18 g, 2.4 mmol) were reduced with 209.1 mg of LAH as a mixture as described above (**10**) to afford 1.04 g of **15a** and **15b** (1.04 g, 96% yield). The two alcohols were isolated by silica gel chromatography with 19:1-9:1 hexanes/EtOAc. **(23S)-6 β -Methoxy-3 α ,5 α -cyclo-23-homoergost-17(20),24(28)-dien-29-ol (15a)**. ¹H NMR (600 MHz): 4.951 (1H, s), 4.815 (1H, s), 3.557 (2H, m), 3.339 (3H, s), 2.790 (1H, t, *J* = 2.8 Hz), 1.743 (3H, m), 1.059 (3H, d, *J* = 6.8 Hz), 1.056 (3H, d, *J* = 6.8 Hz)

,1.028 (3H, s), 0.898 (3H, s), 0.655 (1H, m), 0.441 (1H, dd, $J = 5.2, 8.0$ Hz). ^{13}C NMR (151 MHz): 157.9, 145.8, 122.8, 107.2, 82.4, 65.4, 56.6, 56.5, 47.8, 44.9, 44.4, 43.4, 40.5, 38.2, 35.2, 35.0, 34.3, 33.3, 30.03, 30.01, 25.0, 24.3, 23.1, 22.2, 21.4, 19.2, 18.0, 16.5, 13.1. **(23S)-6 β -Methoxy-3 α ,5 α -cyclo-(22- ^{13}C)-23-homoergost-17(20),24(28)-dien-29-ol (15a*)**. ^1H NMR (600 MHz): 4.954 (1H, s), 4.817 (1H, s), 3.564 (2H, m), 3.337 (3H, s), 2.792 (1H, t, $J = 2.8$ Hz), 1.745 (3H, m), 1.061 (3H, d, $J = 6.8$ Hz), 1.058 (3H, d, $J = 6.8$ Hz), 1.030 (3H, s), 0.900 (3H, s), 0.657 (1H, m), 0.443 (1H, dd, $J = 5.2, 8.0$ Hz). **(23R)-6 β -Methoxy-3 α ,5 α -cyclo-23-homoergost-17(20),24(28)-dien-23-ol (15b)**. ^1H NMR (600 MHz): 4.963 (1H, s), 4.830 (1H, s), 3.562 (2H, m), 3.339 (3H, s), 2.792 (1H, t, $J = 2.8$ Hz), 1.728 (3H, m), 1.062 (3H, d, $J = 6.8$ Hz), 1.056 (3H, d, $J = 6.8$ Hz), 1.031 (3H, s), 0.902 (3H, s), 0.659 (1H, m), 0.444 (1H, dd, $J = 5.2, 8.0$ Hz). ^{13}C NMR (151 MHz): 157.8, 145.6, 122.6, 107.4, 82.4, 65.2, 56.6, 56.3, 47.9, 45.0, 44.9, 43.4, 39.9, 37.9, 35.2, 35.1, 34.1, 33.3, 30.0, 29.9, 25.0, 24.3, 23.1, 22.3, 21.4, 19.2, 17.7, 16.8, 13.1. **(23R)-6 β -Methoxy-3 α ,5 α -cyclo-(22- ^{13}C)-23-homoergost-17(20),24(28)-dien-29-ol (15b*)**. ^1H NMR (600 MHz): 4.963 (1H, s), 4.830 (1H, s), 3.562 (2H, m), 3.339 (3H, s), 2.793 (1H, t, $J = 2.8$ Hz), 1.728 (3H, m), 1.062 (3H, d, $J = 6.8$ Hz), 1.056 (3H, d, $J = 6.8$ Hz), 1.031 (3H, s), 0.902 (3H, s), 0.658 (1H, m), 0.445 (1H, dd, $J = 5.2, 8.0$ Hz). ^{13}C NMR (151 MHz): 39.9 (C22).

(23R)-6 β -Methoxy-3 α ,5 α -cyclo-23-methylergost-17(20),24(28)-diene (16b). To a solution of the alcohol **15b** (749.8 mg, 1.700 mmol) and TEA (1.2 mL, 8.7 mmol) in 10 mL anhydrous DCM at 0 °C was added MsCl (300 μL , 3.9 mmol) dropwise. The resulting solution was stirred for 30 min before extraction with 5% HCl and 4:1 hexanes/EtOAc. The combined organic layers were washed with sodium bicarbonate and then brine, dried over Na_2SO_4 and concentrated under reduced pressure to provide 830.8 mg of the mesylate **24b** (omitted in the scheme). **(23R)-23-[(Methylsulfonyl)oxy]methyl]-6 β -methoxy-3 α ,5 α -cyclo-ergost-17(20),24(28)-diene (24b)**. ^1H NMR (600 MHz): 4.962 (1H, s), 4.856 (1H, s), 4.127 (2H, m), 3.333 (3H, s), 2.959 (3H, s), 2.788 (1H, t, $J = 2.7$ Hz), 1.711 (3H, m), 1.042 (3H, d, $J = 6.8$ Hz), 1.039 (3H, d, $J = 6.8$ Hz), 1.027 (3H, s), 0.895 (3H, s), 0.654 (1H, m), 0.441 (1H, dd, $J = 5.1, 8.0$ Hz). ^{13}C

NMR (151 MHz): 155.9, 146.0, 121.4, 108.2, 8.3, 72.5, 56.6, 56.3, 47.8, 45.0, 43.4, 41.5, 39.7, 37.9, 37.4, 35.2, 35.0, 34.6, 33.3, 30.0, 24.9, 24.3, 23.1, 22.0, 21.8, 21.4, 19.2, 17.9, 16.7, 13.1. The mesylate was reduced with 253.2 mg of LAH as described above (see compound **10**) to afford 598.5 mg (83% yield from of **16b**, 85% yield based on recovered starting material). **(23R)-6 β -Methoxy-3 α ,5 α -cyclo-23-methylergost-17(20),24(28)-diene (16b)**. ¹H NMR (600 MHz): 4.757 (1H, br s), 4.753 (1H, br s), 3.341 (3H, s), 2.794 (1H, t, *J* = 2.8 Hz), 1.690 (3H, m), 1.040 (3H, d, *J* = 6.8 Hz), 1.034 (3H, s), 1.029 (3H, d, *J* = 6.8 Hz), 0.984 (3H, d, *J* = 6.9 Hz), 0.908 (3H, s), 0.658 (1H, m), 0.443 (1H, dd, *J* = 5.2, 8.0 Hz). ¹³C NMR (151 MHz): 162.6, 144.7, 123.3, 104.5, 82.4, 56.6, 56.4, 47.9, 44.9, 44.0, 43.4, 38.0, 36.8, 35.2, 35.1, 33.5, 33.3, 30.0, 29.9, 25.0, 24.3, 23.1, 22.8, 22.5, 21.4, 20.0, 19.2, 17.6, 16.8, 13.1.

(23R)-6 β -Methoxy-3 α ,5 α -cyclo-(22-¹³C)-23-methylergost-17(20),24(28)-diene (16b*). This compound was obtained as described above from **15b*** and its respective mesylate **22b*** (omitted in schemes). **(23R)-23-[(Methylsulfonyl)oxy]methyl]-6 β -methoxy-3 α ,5 α -cyclo-(22-¹³C)Cergost-17(20),24(28)-diene (22b*)**. ¹H NMR (600 MHz): 4.964 (1H, s), 4.858 (1H, s), 4.127 (2H, m), 3.335 (3H, s), 2.961 (3H, s), 2.790 (1H, t, *J* = 2.7 Hz), 1.713 (3H, m), 1.044 (3H, d, *J* = 6.8 Hz), 1.041 (3H, d, *J* = 6.8 Hz), 1.028 (3H, s), 0.897 (3H, s), 0.657 (1H, m), 0.443 (1H, dd, *J* = 5.2, 8.0 Hz). ¹³C NMR (151 MHz): 39.7 (C22). **(23R)-6 β -Methoxy-3 α ,5 α -cyclo-(22-¹³C)-23-methylergost-17(20),24(28)-diene (16b*)**. ¹H NMR (600 MHz): 4.758 (1H, br s), 4.754 (1H, br s), 3.341 (3H, s), 2.796 (1H, t, *J* = 2.8 Hz), 1.692 (3H, m), 1.042 (3H, d, *J* = 6.8 Hz), 1.036 (3H, s), 1.029 (3H, d, *J* = 6.8 Hz), 0.984 (3H, d, *J* = 6.9 Hz), 0.908 (3H, s), 0.6598 (1H, m), 0.443 (1H, dd, *J* = 5.2, 8.0 Hz). ¹³C NMR (151 MHz): 44.0 (C22).

(23S)-6 β -Methoxy-3 α ,5 α -cyclo-23-methylergost-17(20),24(28)-diene (16a). This compound was obtained as described above from **15a** and its respective mesylate **24a** (omitted in scheme). **(23S)-23-[(Methylsulfonyl)oxy]methyl]-6 β -methoxy-3 α ,5 α -cyclo-ergost-17(20),24(28)-diene (22a)**. ¹H NMR (600 MHz): 4.967 (1H, s), 4.861 (1H, s), 4.137 (2H, d, *J* = 6.7 Hz), 3.334 (3H, s), 2.956 (3H, s), 2.792 (1H, t, *J* = 2.8 Hz), 1.715 (3H, m), 1.044 (3H, d, *J* = 6.8 Hz), 1.043 (3H, d, *J* = 6.8 Hz), 1.027 (3H, s),

0.885 (3H, s), 0.655 (1H, m), 0.442 (1H, dd, $J = 5.2, 8.0$ Hz). ^{13}C NMR (151 MHz): 156.2, 146.2, 121.3, 108.0, 82.4, 72.1, 56.6, 56.4, 47.7, 44.9, 43.4, 41.1, 40.0, 38.0, 37.4, 35.2, 35.0, 34.6, 33.3, 30.01, 30.0, 24.9, 24.3, 23.1, 21.9, 21.7, 21.4, 19.2, 18.0, 16.5, 13.1. **(23S)-6 β -Methoxy-3 α ,5 α -cyclo-23-methylergosta-17(20),24(28)-diene (16a).** ^1H NMR (600 MHz): 4.761 (1H, br s), 4.752 (1H, s), 3.338 (3H, s), 2.792 (1H, t, $J = 2.8$ Hz), 1.691 (3H, m), 1.040 (3H, d, $J = 6.8$ Hz), 1.035 (3H, s), 1.031 (3H, d, $J = 6.8$ Hz), 0.966 (3H, d, $J = 6.9$ Hz), 0.905 (3H, s), 0.656 (1H, m), 0.443 (1H, dd, $J = 5.2, 8.0$ Hz). ^{13}C NMR (151 MHz): 162.7, 144.8, 123.1, 104.4, 82.4, 56.6, 56.59, 47.9, 44.8, 44.3, 43.4, 38.2, 36.3, 35.3, 35.0, 33.6, 33.3, 30.04, 33.0, 25.0, 24.3, 23.1, 22.7, 22.4, 21.4, 19.5, 19.2, 17.9, 16.6, 13.1.

(23R)-23-Methylergost-17(20),24(28)-dien-3 β -ol (17b). To a solution of **16b** (344.2 mg, 0.81 mmol) in 5 mL of anhydrous DCM was added 105 μL of TFA. The resulting solution was stirred at room temperature for 30 min before adding 3 mL of 4:1 MeOH/TEA dropwise. The mixture was heated at 45 $^\circ\text{C}$ for 1h before removing the solvent under reduced pressure. The obtained crude solid was purified via silica gel chromatography with 9:1 hexanes/EtOAc to afford the desired product **17b** as a white crystal (250.7 mg, 75% yield). ^1H NMR (600 MHz): 5.367 (1H, m), 4.756 (2H, m), 3.527 (1H, m), 1.695 (3H, m), 1.036 (3H, d, $J = 6.7$ Hz), 1.025 (3H, d, $J = 6.7$ Hz), 1.019 (3H, s), 0.984 (3H, d, $J = 6.9$ Hz), 0.876 (3H, s). ^{13}C NMR (151 MHz): 162.6, 144.5, 140.8, 123.7, 121.6, 104.6, 71.8, 56.6, 50.0, 44.0, 42.3, 37.5, 37.2, 36.8, 36.5, 33.5, 31.8, 31.7, 31.4, 29.9, 24.5, 22.7, 22.5, 21.5, 20.0, 19.4, 17.6, 16.4.

(23R)-(22- ^{13}C)23-methylergost-17(20),24(28)-dien-3 β -ol (17b*) from 16b*. ^1H NMR (600 MHz): 5.367 (1H, m), 4.756 (2H, br s), 3.527 (1H, m), 1.695 (3H, m), 1.036 (3H, d, $J = 6.7$ Hz), 1.025 (3H, d, $J = 6.7$ Hz), 1.019 (3H, s), 0.983 (3H, dd, $J = 4.7$ (J_{CH}), 6.9 Hz), 0.876 (3H, s). ^{13}C NMR (151 MHz): 44.0 (C22).

(23S)-23-Methylergost-17(20),24(28)-dien-3 β -ol (17a) from 16a. ^1H NMR (600 MHz): 5.367 (1H, m), 4.761 (1H, m), 4.752 (1H, m), 3.529 (1H, m), 1.696 (3H, m), 1.038 (3H, d, $J = 6.7$ Hz), 1.029 (3H, d, $J =$

6.8 Hz), 1.020 (3H, s), 0.966 (3H, d, $J = 6.9$ Hz), 0.871 (3H, s). ^{13}C NMR (151 MHz): 162.7, 144.6, 140.8, 123.5, 121.6, 104.4, 71.8, 56.8, 50.0, 44.4, 44.3, 42.3, 37.7, 37.2, 36.5, 36.3, 33.6, 31.8, 31.7, 31.3, 30.0, 24.5, 22.7, 22.4, 21.5, 19.6, 19.3, 18.0, 16.1.

(23R)-23-Methylergost-4,17(20),24(28)-trien-3-one (18b). To a solution of the alcohol **17b** (200.5 mg, 0.490 mmol) and $\text{Al}(\text{iPrO})_3$ (400.8 mg, 1.960 mmol) of in 10 mL of anhydrous toluene was added anhydrous 3-methyl-2-butanone (2.5 mL, 23.3 mmol). The mixture was stirred at reflux under a N_2 atmosphere for 16 h. The reaction was cooled to room temperature before extraction with 10% hydrochloric acid. The combined organic layers were washed with sodium bicarbonate and then brine, dried over Na_2SO_4 and concentrated under reduced pressure. The desired product was obtained as white solid after purification with silica gel chromatography with 19:1 hexanes/EtOAc (169.1 mg, 85% yield). ^1H NMR (600 MHz): 5.731 (1H, s), 4.760 (1H, br s), 4.751 (1H, br s), 1.690 (3H, m), 1.191 (3H, s), 1.034 (3H, d, $J = 6.5$ Hz), 1.024 (3H, d, $J = 6.5$ Hz), 0.984 (3H, d, $J = 6.9$ Hz), 0.903 (3H, s). ^{13}C NMR (151 MHz): 199.6, 171.5, 162.5, 144.0, 124.0, 123.8, 104.6, 55.7, 53.6, 44.4, 44.0, 38.6, 37.4, 36.8, 35.6, 35.1, 34.0, 33.5, 32.9, 31.9, 29.8, 24.3, 22.7, 22.5, 21.4, 20.1, 17.6, 17.3, 16.5.

(23S)-23-Methylergost-4,17(20),24(28)-trien-3-one (18a) from 17a. ^1H NMR (600 MHz): 5.731 (1H, s), 4.763 (1H, s), 4.750 (1H, s), 1.691 (3H, m), 1.192 (3H, s), 1.038 (3H, d, $J = 6.5$ Hz), 1.029 (3H, d, $J = 6.5$ Hz), 0.961 (3H, d, $J = 6.9$ Hz), 0.898 (3H, s). ^{13}C NMR (151 MHz): 199.6, 171.5, 162.6, 144.0, 123.8 (2C), 104.5, 55.9, 53.6, 44.4, 44.3, 8.6, 37.6, 36.3, 35.7, 35.1, 34.0, 33.6, 32.9, 31.9, 29.9, 24.3, 22.7, 22.4, 21.4, 19.6, 17.9, 17.3, 16.3.

(23S)-4 α ,23-Dimethylergost-17(20),24(28)-dien-3-one (19a) and (23S)-2 α ,4 α ,23-trimethylergost-17(20),24(28)-dien-3-one 20a. To a solution of Li (190.3 mg, 27.6 mmol) in 15 mL of NH_3 in an oven-dry two-neck flask equipped with a drying tube was added dropwise the dissolved enone **18a** (171.1 mg, 0.42 mmol) in 7 mL of dry anhydrous THF. After 1.5 h, 450 μL of anhydrous isoprene was slowly added

followed 250 μ L of anhydrous HMPA. The mixture was cooled to -78 $^{\circ}$ C before the dropwise addition of MeI (250 μ L, 4.0 mmol). The NH_3 was allowed to evaporate overnight through a drying tube. The reaction was extracted with 10% hydrochloride and 4:1 hexanes/EtOAc. The combined organic layers were washed with sodium bicarbonate and then brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by silica gel chromatography with 39:1 hexanes/EtOAc to afford of the desired product **19a** (78.3 mg, 44% yield) and 32.8 mg of **20a**. Both products were obtained as a white solid. **(23S)-4 α ,23-Dimethylergost-17(20),24(28)-dien-3-one (19a)**. ^1H NMR (600 MHz): 4.759 (1H, br s), 4.748 (1H, br s), 2.441(1H, m), 1.682 (3H, m), 1.076 (3H, s), 1.036 (3H, d, $J = 6.7$ Hz), 1.027 (3H, d, $J = 6.8$ Hz), 0.981 (3H, d, $J = 6.5$ Hz), 0.960 (3H, d, $J = 6.9$ Hz), 0.864 (3H, s). ^{13}C NMR (151 MHz): 213.6, 162.7, 144.5, 123.4, 104.4, 56.3, 53.9, 53.6, 45.1, 44.5, 44.4, 39.2, 38.0, 37.9, 36.4, 36.3, 34.3, 33.6, 31.8, 30.0, 26.5, 24.3, 22.7, 22.4, 21.8, 19.6, 17.9, 16.4, 12.6, 11.5. **(23S)-2 α ,4 α ,23-Trimethylergost-17(20),24(28)-dien-3-one 20a**. ^1H NMR (600 MHz): 4.759 (1H, s), 4.748 (1H, s), 2.522 (1H, s), 1.682 (3H, br s), 1.126 (3H, s), 1.036 (3H, d, $J = 6.5$ Hz), 1.027 (3H, d, $J = 6.5$ Hz), 1.001 (3H, d, $J = 6.5$ Hz), 0.956 (6H, d, $J = 6.5$ Hz), 0.864 (3H, s).

(23R)-4 α ,23-Dimethylergost-17(20),24(28)-dien-3-one (19b) and (23R)-2 α ,4 α ,23-trimethylergost-17(20),24(28)-dien-3-one (20b) from 18b. ^1H NMR (600 MHz): 4.756 (1H, br s), 4.749 (1H, br s), 2.441(1H, m), 1.681 (3H, m), 1.074 (3H, s), 1.034 (3H, d, $J = 6.7$ Hz), 1.023 (3H, d, $J = 6.7$ Hz), 0.981 (6H, d, $J = 6.7$ Hz), 0.864 (3H, s). ^{13}C NMR (151 MHz): 213.7, 162.5, 144.5, 123.6, 104.6, 56.1, 53.9, 53.6, 45.1, 44.6, 44.0, 39.2, 38.0, 37.7, 36.8, 36.4, 34.3, 33.5, 31.8, 29.9, 25.6, 24.3, 22.7, 22.5, 21.8, 20.0, 17.6, 16.6, 12.7, 11.5. **(23R)-2 α ,4 α ,23-trimethylergost-17(20),24(28)-dien-3-one (20b)**. ^1H NMR (600 MHz): 4.756 (1H, s), 4.749 (1H, s), 2.520 (1H, s), 1.680 (3H, br s), 1.124 (3H, s), 1.033 (3H, d, $J = 6.6$ Hz), 1.022 (3H, d, $J = 6.6$ Hz), 0.999 (3H, d, $J = 6.5$ Hz), 0.979 (3H, d, $J = 6.9$ Hz), 0.961 (6H, d, $J = 6.6$ Hz), 0.868 (3H, s). ^{13}C NMR (151 MHz): 214.7, 162.5, 144.5, 123.6, 104.6, 56.1, 54.6, 54.0, 49.2, 44.7,

44.6, 44.0, 40.7, 37.7, 36.8, 36.78, 34.3, 33.5, 31.9, 29.9, 25.5, 24.3, 22.7, 22.5, 21.8, 20.0, 17.6, 16.6, 14.9, 13.6, 11.5.

(23R)-4 α -(¹³C-Methyl)-23-methylergost-17(20),24(28)-dien-3-one (19b*) and (23R)-2 α ,4 α -(¹³C-dimethyl)-23-methylergost-17(20),24(28)-dien-3-one (20b*) from 18b. ¹H NMR (600 MHz): 4.755 (1H, br s), 4.748 (1H, br s), 2.441 (1H, m), 1.682 (3H, br s), 1.074 (3H, s), 1.034 (3H, d, *J* = 6.7 Hz), 1.023 (3H, d, *J* = 6.7 Hz), 0.980 (3H, d, *J* = 6.8 Hz), 0.980 (3H, dd, *J* = 6.5, 126.1 (*J*_{CH}) Hz), 0.869 (3H, s). ¹³C NMR (151 MHz): 11.5 (4 α -Me). **(23R)-2 α ,4 α -(¹³C-dimethyl)-23-methylergost-17(20),24(28)-dien-3-one (20b*).** ¹H NMR (600 MHz): 4.756 (1H, s), 4.749 (1H, s), 2.518 (1H, s), 1.680 (3H, m), 1.123 (3H, s), 1.033 (3H, d, *J* = 6.6 Hz), 1.022 (3H, d, *J* = 6.6 Hz), 0.998 (3H, dd, *J* = 6.5, 126.8 (*J*_{CH}) Hz), 0.979 (3H, d, *J* = 6.9 Hz), 0.960 (6H, d, *J* = 6.5, 127.0 (*J*_{CH}) Hz), 0.868 (3H, s). ¹³C NMR (151 MHz): 14.9 (2 α -Me), 11.5 (4 α -Me).

(23S)-4 α ,23-Dimethylergost-17(20),24(28)-dien-3 β -ol (23-epipfiesterol, 3). To a solution of Li (70.5 mg, 10.2 mmol) in 10 mL of NH₃ was added of **19a** (73.3 mg, 0.17 mmol) in 2 mL of Et₂O. After 1 h, 250 μ L of tBuOH was added. After allowing the NH₃ to evaporate at room temperature, the reaction was extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The combined organic layers were washed with sodium bicarbonate and then brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by silica gel chromatography with 9:1 hexanes/EtOAc to afford compound **3** as a white solid (52.6 mg, 76% yield). ¹H NMR (600 MHz): 4.756 (1H, br s), 4.747 (1H, br s), 3.084 (1H, m), 1.679 (3H, m), 1.036 (3H, d, *J* = 6.7 Hz), 1.026 (3H, d, *J* = 6.7 Hz), 0.962 (3H, d, *J* = 6.9 Hz), 0.955 (3H, d, *J* = 6.4 Hz), 0.836 (6H, s). ¹³C NMR (151 MHz): 162.7, 144.8, 123.2, 104.4, 76.6, 56.6, 54.4, 51.0, 44.5, 44.4, 39.2, 38.0, 36.8, 36.3, 36.0, 34.3, 33.6, 23.1, 31.1, 30.0, 24.3, 24.2, 22.7, 22.4, 21.5, 19.6, 17.9, 16.4, 15.1, 13.3.

(23R)-4 α ,23-Dimethylergost-17(20),24(28)-dien-3 β -ol (Pfisterol, 1) from 19b. ¹H NMR (600 MHz): 4.754 (1H, br s), 4.749 (1H, br s), 3.083 (1H, m), 1.678 (3H, m), 1.034 (3H, d, *J* = 6.8 Hz), 1.023 (3H, d, *J* = 6.8 Hz), 0.977 (3H, d, *J* = 6.9 Hz), 0.955 (3H, d, *J* = 6.4 Hz), 0.840 (3H, s), 0.836 (3H, s). ¹³C NMR (151 MHz): 162.6, 144.7, 123.4, 104.5, 76.6, 56.3, 54.4, 50.9, 44.6, 44.0, 39.2, 37.8, 36.8, 36.7, 36.0, 34.3, 33.5, 32.1, 31.1, 29.9, 24.3, 24.2, 22.7, 22.5, 21.5, 20.0, 17.5, 16.6, 15.1, 13.3.

(23R)-4 α -(¹³C-Methyl)-23-methylergost-17(20),24(28)-dien-3 β -ol (1*) from 19b*. ¹H NMR (600 MHz): 4.755 (1H, br s), 4.751 (1H, br s), 3.084 (1H, m), 1.679 (3H, m), 1.036 (3H, d, *J* = 6.8 Hz), 1.024 (3H, d, *J* = 6.8 Hz), 0.978 (3H, d, *J* = 6.9 Hz), 0.954 (3H, dd, *J* = 6.4, 125.1 (*J*_{CH}) Hz), 0.840 (3H, s), 0.836 (3H, s). ¹³C NMR (151 MHz): 15.1 (4 α -Me).

(23S)-4-Phenylthiomethyl-23-methylergost-4,17(20),24(28)-trien-3-one (21a). To a solution of **18a** (25.8 mg, 0.0600 mmol) in 700 μ L of MeOH in a stretched glass culture tube was added 150 μ L of thiophenol, 250 μ L of formalin (37% formaldehyde w/w) and 37 μ L of TEA. The tube was flame sealed and the mixture was heated at 120 °C for 19 h. The reaction was allowed to cool to room temperature before extraction with 9:1 hexanes/EtOAc. The combined organic layers were washed with sodium bicarbonate and then brine, dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified via silica gel chromatography with 19:1 hexanes/EtOAc to obtain compound **21a** (17.2 mg, 51% yield). ¹H NMR (600 MHz): 7.387 (2H, m), 7.187 (1H, m), 4.764 (1H, br s), 4.751 (1H, br s), 3.881 (2H, br s), 2.722 (1H, m), 1.687 (3H, m), 1.165 (3H, s), 1.038 (3H, d, *J* = 6.8 Hz), 1.029 (3H, d, *J* = 6.8 Hz), 0.963 (3H, d, *J* = 6.9 Hz), 0.885 (3H, s). ¹³C NMR (151 MHz): 197.0, 168.2, 162.6, 144.0, 136.6, 131.0 (2 C), 128.7 (2 C), 128.3, 126.5, 123.8, 104.5, 55.9, 54.0, 44.4, 44.3, 39.5, 37.6, 36.3, 34.8, 34.6, 33.7, 33.6, 32.0, 29.9, 28.9, 28.2, 24.3, 22.7, 22.4, 21.4, 19.7, 17.9, 17.85, 16.3.

(23R)-4-Phenylthiomethyl-23-methylergost-4,17(20),24(28)-trien-3-one (21b) from 18b. ¹H NMR (600 MHz): 7.386 (2H, m), 7.188 (1H, m), 4.760 (1H, br s), 4.751 (1H, br s), 3.877 (2H, s), 2.722 (1H,

m), 1.682 (3H, br s), 1.160 (3H, s), 1.035 (3H, d, $J = 6.8$ Hz), 1.024 (3H, d, $J = 6.8$ Hz), 0.982 (H, d, $J = 6.9$ Hz), 0.886 (3H, s). ^{13}C NMR (151 MHz): 197.0, 168.2, 162.5, 143.9, 136.5, 131.0 (2 C), 128.7 (2 C), 128.2, 126.5, 123.9, 104.6, 55.6, 54.0, 44.4, 44.0, 37.4, 36.8, 34.7, 34.6, 33.6, 33.5, 31.9, 29.8, 28.9, 28.2, 24.2, 22.7, 22.5, 21.4, 20.1, 17.8, 17.5, 16.5.

Pfiesterol (1) from 21b. To a solution of Li (61.2 mg, 8.80 mmol) of in 15 mL of NH_3 was added compound **21b** (12.6 mg, 0.0200 mmol) of in 2 mL of Et_2O . The mixture was stirred for 30 min before adding 400 μL of MeOH dropwise. After 45 min, additional Li (35.1 mg, 5.1 mmol) was added to the reaction. After 1 h, NH_3 (5 mL) was added and the reaction was left to stir overnight. The residue obtained after the NH_3 slowly evaporated overnight was extracted with 4:1 hexanes/EtOAc and 10% hydrochloric acid. The combined organic layers were washed with sodium bicarbonate and then brine, dried over Na_2SO_4 and concentrated under reduced pressure. The product **1** was purified via preparative TLC with 4:1 hexanes/EtOAc (8.1 mg, 80% yield).

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CHAPTER 4

Synthesis and NMR Characterization of 4-Methylcholestane-3 β ,4 β -diol, a Pavlovol, from Cholestenone

Abstract:

The synthesis of 4-methylcholestane-3 β ,4 β -diol, a synthetic pavlovol, from cholestenone in a seven-step sequence and a 38% overall yield is reported. Pavlovols in cultured *Pavlova* spp. were found to behave as analogs of the hormone ecdysone in northern bay scallop larvae (*Argopecten irradians irradians*). Another route to 4-methylcholestane-3 β ,4 β -diol from 4-methylencholestanol is also described. All the products were characterized by NMR. The NMR characterization of (24R)-4-methylstigmastane-3 β ,4 β -diol and (24R)-4-methylsigmast-22-en-3 β ,4 β -diol are also reported. These sterols provided reference compounds and samples for biological testing of their effects on the growth and development of scallop larvae.

4.1 Introduction

Pavlovols, 3 β ,4 β -dihydroxy-4-methylsterols, are natural sterols that have been exclusively isolated in microalgae belonging to the genera *Pavlova* and *Diacronema*.¹⁻² Both genera are part of the family *Pavlovaceae* and the order Pavloales, hence the name of those dihydroxy sterols that are also

considered biomarkers for that order.³ Four natural pavlovols have been reported to date: methylpavlovol (**1**), ethylpavlovol (**2**), 22-dehydromethylpavlovol (**3**) and 22-dehydroethylpavlovol (**4**) (Figure 4.1).¹⁻³

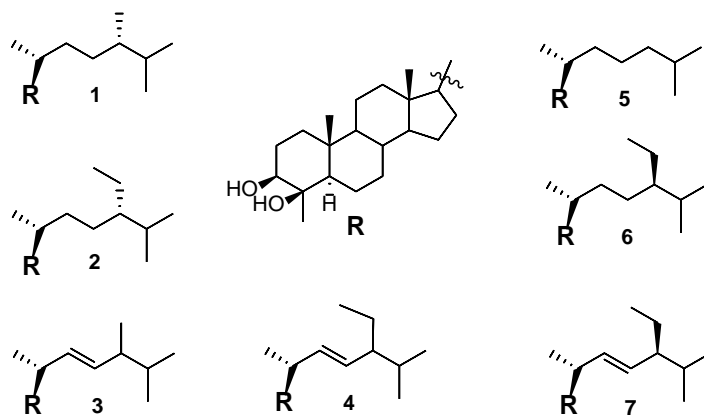
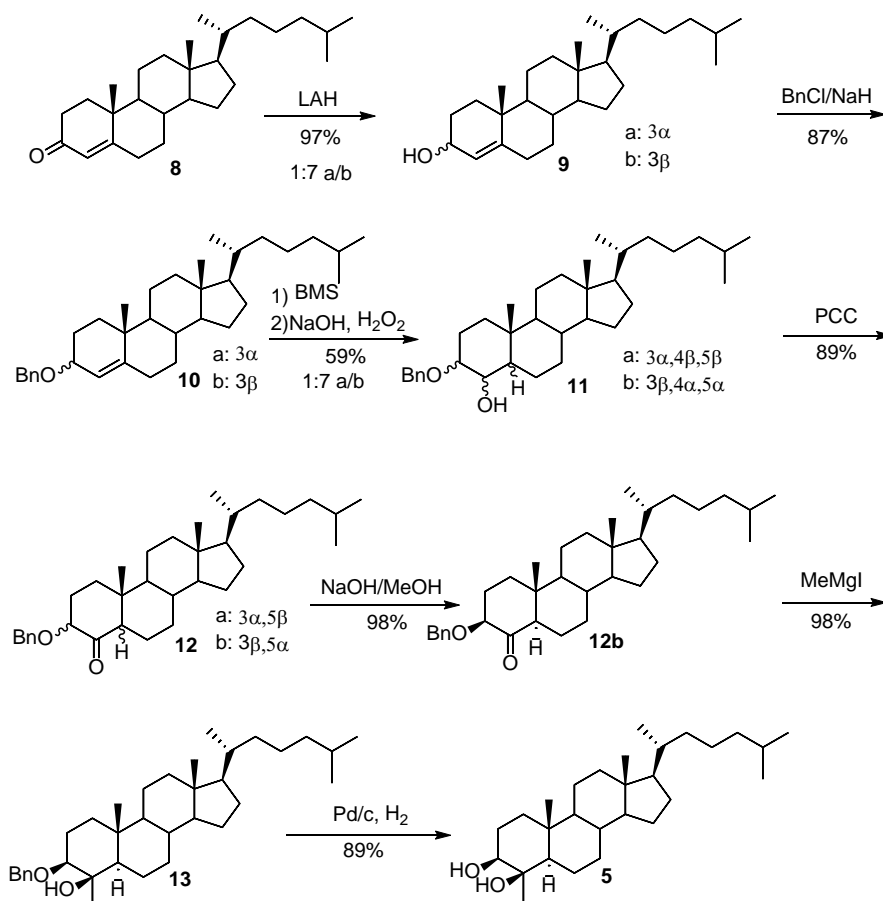


Figure 4.1. The natural pavlovols (**1-4**) and the reported synthetic pavlovols (**5-7**).

Microalgae, including some *Pavlova* species, have been extensively used in aquaculture as live foods for bivalves because of their high content of essential nutrients.^{1,2,4} Microalgae are the primary source of sterols for marine invertebrates and shellfishes, as they are either no longer able to synthesize or transform their own essential sterols or do so poorly.⁵⁻⁷ Pavlovols in cultured *Pavlova* spp. were found to behave as analogs of the hormone ecdysone by inducing early metamorphosis in northern bay scallop (*Argopecten irradians irradians*) larvae and to be responsible for settlement induction.⁸ A pavlovol with the cholesterol side chain (**5**) was made in a seven-step sequence from cholestenone with a 38% overall yield. Two other synthetic pavlovols made from stigmasterol, (24R)-4-methylstigmastane-3 β ,4 β -diol (**6**) and (24R)-4-methylstigmast-22-en-3 β ,4 β -diol (**7**), are also described. These sterols offer new material for testing on the effects of pavlovols on bivalves. Another method to 4-methylcholestane-3 β ,4 β -diol from 4-methylenecholestanol is also described.

4.2 Results and Discussion

The synthesis of 4-methylcholestane-3 β ,4 β -diol (**5**) from cholestenone is summarized in Scheme 4.1. Cholestenone (**8**), which is commercially available and can easily be made from cholesterol⁹, was reduced with LAH to obtain a 7:1 mixture of the allylic alcohols **10** favoring the 3 β product in near quantitative yield. The mixture of alcohols was benzylated with benzyl bromide and the product mixture **10** was converted to the mixture of alcohols **11** via a hydroboration-oxidation reaction¹⁰. Heating the oxidation reaction at 50 °C overnight was increased the reaction yield, but still the yield was only a modest 59 %. A 7:1 mixture of the alcohols **11b/11a**, was obtained from the reaction. Heating the oxidation reaction at 50 °C overnight was increased the reaction yield, but still the yield was only a modest 59 %. A 7:1 mixture of the alcohols **11b/11a**, was obtained from the reaction.



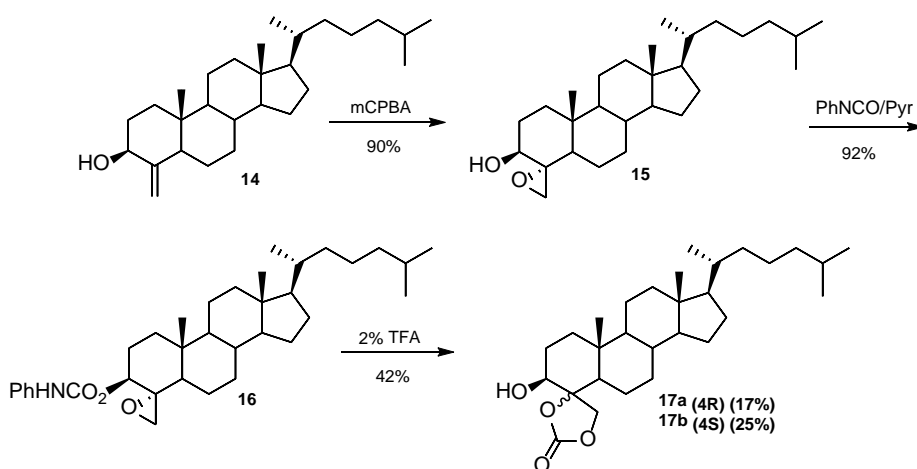
Scheme 4.1. Synthesis of compound **5** from cholestenone **8**.

The mixture of alcohols **11a** and **11b** from the hydroboration-oxidation step were oxidized to their corresponding ketones **12** with PCC in good yield (89%). It was found that the mixture of the ketones **12a** and **12b** could be isomerized with methanolic NaOH at reflux to obtain the pure desired ketone **12b** in near quantitatively yield. The epimerization of the ketone mixture to one product **12b** boosted the sequence overall yield by salvaging the undesired isomer **12a** which originated from cholest-4-en-3 α -ol (**9a**), the minor product of the LAH reduction of cholestenone in the very first step of the sequence. The conversion of the ketone mixture to one product **12b** also provided a direct path to the final product **5** without the need for purification by chromatography. The ketone **12b** was selectively alkylated with methylmagnesium iodide to afford only compound **13** in a 98% yield. The selectivity the alkylation reaction can be attributed to steric hindrance over the top face of the carbonyl by the benzyl ether at C3 and the methyl group at C10. The desired synthetic pavlovol, 4-methylcholestane-3 β ,4 β -diol **5**, was obtained from the deprotection of the benzyl ether via catalytic hydrogenation in an 89 % yield from intermediate **12**, and a 38% overall yield from cholestenone.

The final product, 4-methylcholestane-3 β ,4 β -diol (**5**), was confirmed as a pavlovol by comparing its NMR data with the ^1H and ^{13}C data^{1b} reported for methylpavlovol (**1**) and ethylpavlovol (**2**) (Table 4.1). Comparison of the nuclear ^1H and ^{13}C signals of compound **5** and methylpavlovol and ethylpavlovol indicated that all three compounds have a similar nucleus. Only minor differences (under 10 ppb) were observed between the signals of the nucleus. The side chain ^1H and ^{13}C chemical shifts for the three compounds only had similar values for C20 and C21. The NMR data for compounds **6** and **7** are reported in the experimental section.

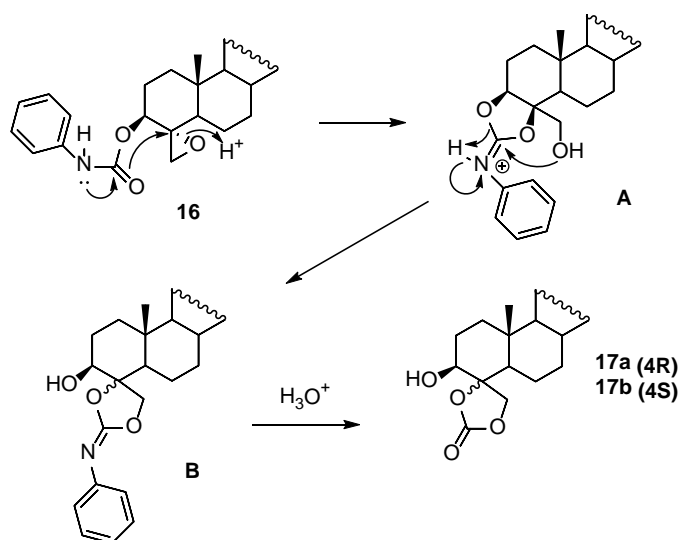
Table 4.1. ^1H and ^{13}C chemical shifts (ppm) for compounds **1**^{1b}, **2**^{1b} and **5**. *30: 4-methyl carbon.

Carbon #	^{13}C shifts			^1H shifts		
	1	2	5	1	2	5
1	36.68	36.61	36.64			
2	27.21	27.17	27.17			
3	75.69	75.65	75.66	3.249	3.250	3.247
4	74.18	74.17	74.16			
5	53.00	52.92	52.95			
6	20.62	20.60	20.59			
7	32.52	32.48	32.49			
8	34.96	34.90	34.91			
9	55.67	55.59	55.62	0.59	0.59	0.581
10	36.26	36.22	36.22			
11	20.64	20.60	20.61			
12	40.02	39.97	40.00			
13	42.58	42.53	42.55			
14	56.66	56.61	56.64			
15	24.21	24.19	24.17			
16	28.25	28.28	28.27			
17	56.16	56.12	56.27			
18	12.10	12.07	12.07	0.643	0.646	0.646
19	13.99	13.97	13.97	1.006	1.009	1.009
20	36.21	36.28	36.186			
21	18.86	18.77	18.66	0.899	0.906	0.895
22	33.76	33.89	35.78			
23	30.65	26.37	23.84			
24	39.13	46.05	39.52			
25	31.52	28.93	28.00			
26	17.64	19.60	22.55	0.779	0.828	0.863
27	20.51	13.97	22.80	0.771	0.807	0.859
28	15.48	26.37				
29		12.32				
*30	25.37	25.36	25.35			



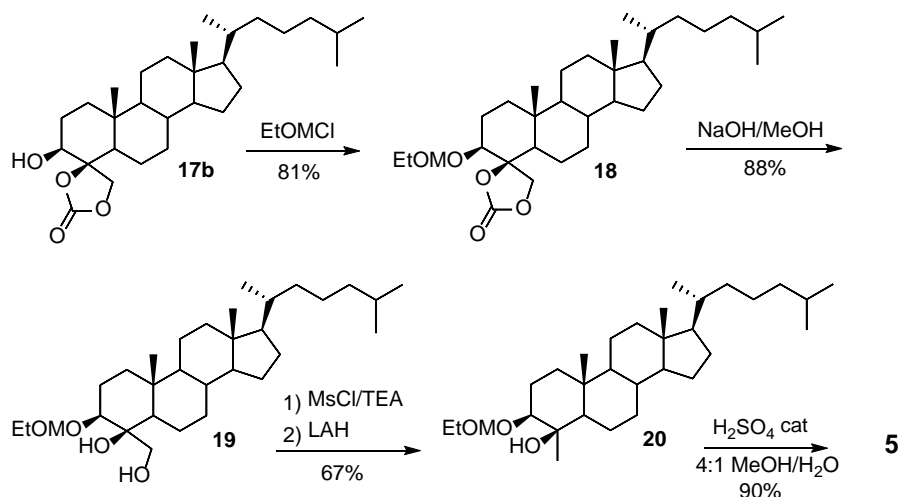
Scheme 4.2. Synthesis of intermediates **17a** and **17b** from 4-methylenecholestanol **15**.

In the second method, compound **5** was made from the known sterol 4-methylenecholestanol **14**¹². The starting material **14** was easily and cleanly converted to the epoxide **15**^{12b-13} with mCPBA (Scheme 4.2). The oxygen was added exclusively to the bottom face of the exo-methylene. The hydroxyl group in this case did not participate in the stereodirection of the epoxidation.^{12b} The epoxy-alcohol was treated with phenyl isocyanate and pyridine in DCM to afford the epoxy-phenylcarbamate **16**.¹⁴ The treatment of intermediate **16** with a 2% TFA solution in anhydrous DCM gave a 2:3 mixture of the cyclic carbonates **17a** and **17b** in 42% yield.^{15,16} Multiple side products were observed from this reaction, but they were not characterized. The desired product **17b** was only obtained in a 25% yield. A proposed mechanism for the formation of the cyclic carbonates is illustrated in Scheme 4.3. In the anhydrous 2% TFA/DCM solution, the phenyl carbamate and the epoxide in compound **15** rearranged to the resonance stabilized iminium hydroxymethyl intermediate **A** that reacted intermolecularly to give the mixture of imines **B**. The cyclic carbonates **17a** and **17b** are readily obtained from the hydrolysis of the imine mixture **B**.



Scheme 4.3. Mechanism for the formation of the cyclic carbonates **17a** and **17b**.

With the desired stereochemistry already in at C4, intermediate **17b** was converted to its corresponding ethoxymethyl ether **18** with chloromethyl ethyl ether (Scheme 4.4).^{15b,17} Compound **19** was obtained by transesterification of the cyclic carbonate with methanolic NaOH at reflux.¹⁸ The 4 α -hydroxymethyl was selectively deoxygenated in a two-step procedure to obtain compound **20**.¹⁹ To achieve the final product **5**, the 3 β -hydroxyl was deprotected with catalytic sulfuric acid in a 4:1 MeOH/H₂O at reflux in a 90% yield. This method was originally considered harsh with the tertiary alcohol at C4. However, no noticeable dehydration product was observed in the crude mixture. The final product was spectrally identical to the pavlovovl generated by the original method.



Scheme 4.4. Synthesis of compound **3** from intermediate **16b**.

4.3 Summary

The synthesis of the unnatural pavlovols, 4-methylcholestane-3 β ,4 β -diol (**5**), was successfully accomplished from cholestenone in seven steps and a 38% overall yield. The final product was characterized by ^1H and ^{13}C NMR. Its structure was confirmed by comparison to NMR data for the natural sterols methylpavlovols (**1**) and ethylpavlovols (**2**). Another seven-step sequence was used to make compound **3** from 4-methylenechlestanol. The second sequence was less effective as only an 8% overall yield was observed. The TFA catalyzed reaction only gave the desired cyclocarbonate in a 25% yield. Two new synthetic pavlovols synthesized from stigmasta-5,22-dien-3-one, (24R)-4-methylstigmastane-3 β ,4 β -diol (**6**) and (24R)-4-methylstigmast-22-en-3 β ,4 β -diol (**7**), are also reported. These sterols are 24 epimers of the natural pavlovols **2** and **4**. All three pavlovols were made available for studies of their biological effects on bivalve growth and development. Pavlovols behave as natural

analogs of ecdysone, a natural invertebrate hormone that is also involved in life-history transitions in bivalve molluscs.⁸

4.4 Experimental

General Procedures. NMR spectra were obtained in CDCl₃ on a Bruker 600 MHz spectrometer (¹H at 600 MHz and ¹³C at 151 MHz). Preparative thin layer chromatography (TLC) purifications were done with Sorbent Technologies (SORBTECH) silica gel HL thin TLC plates (20 x 20 cm², 250 μm thick with glass backed support). All solvents were from commercial sources.

Cholest-4-en-3-ol (9). A solution of LAH (400.6 mg, 10.5 mmol) in 10 mL of anhydrous ether was added dropwise to a stirring mixture of **8** (2.63 g, 6.84 mmol) in 4 mL of anhydrous ether. After 30 min, the reaction was quenched by slow addition brine. The resulting mixture was diluted with 10% Hydrochloric acid and extracted with 4:1 hexanes/EtOAc. The organic layer was washed with saturated sodium bicarbonate, dried over Na₂SO₄, filtered through silica and evaporated under reduced pressure to give of a 6.7:1 mixture of favoring the 3β-product **9b** (2.56 g, 97% yield).

3-Benzoyloxy-cholest-4-ene (10). Benzyl bromide (3.8 mL, 31.9 mmol) was added to a solution of **9** (8.1 g, 20.9 mmol) dissolved in 5 mL of dry THF in a 25-mL flask equipped with a reflux condenser. NaH (4.0 g) was transferred in 3 mL of anhydrous THF to the reaction mixture before heating the resulting solution at reflux under a N₂ atmosphere. After 16 h, the reaction was cooled to rt, filtered through silica gel and eluted with 4:1 hexanes/EtOAc. The crude product was evaporated under reduced pressure. Flash chromatography using 79:1-39:1 hexanes/EtOAc gave a 6.7:1 β/α mixture of **10** (8.65 g, 87%). A sample of the mixture was separated by preparative TLC with 39:1 hexanes/EtOAc. **3α-Benzoyloxy-cholest-4-ene**

(10a). ^1H NMR (600 MHz): 5.455 (1H, m), 4.594 (1H, d, $J = 12.2$ Hz), 4.519 (1H, d, $J = 12.2$ Hz), 3.771 (1H, m), 2.205 (1H, m), 0.977 (3H, s), 0.904 (3H, d, $J = 6.6$ Hz), 0.867 (3H, d, $J = 6.6$ Hz), 0.863 (3H, d, $J = 6.6$ Hz), 0.680 (1H, s). ^{13}C NMR (151 MHz) (3- α product): 150.6, 139.4, 128.3 (2 C), 127.7 (2 C), 127.3, 118.7, 70.9, 70.0, 56.2, 56.1, 53.5, 42.5, 39.9, 39.5, 37.6, 36.2, 35.9, 35.8, 32.7, 32.5, 32.1, 28.2, 28.1, 24.3, 24.2, 23.8, 22.8, 22.6, 21.5, 18.7, 18.2, 12.0. **3 β -Benzyloxy-cholest-4-ene (10b).** ^1H NMR (600 MHz): 5.389 (1H, m), 4.601 (1H, d, $J = 11.9$ Hz), 4.561 (1H, d, $J = 11.9$ Hz), 3.944 (1H, m), 2.200 (1H, m), 1.813 (1H, m), 1.052 (3H, s), 0.901 (3H, d, $J = 6.6$ Hz), 0.866 (3H, d, $J = 6.6$ Hz), 0.861 (3H, d, $J = 6.6$ Hz), 0.677 (1H, s). ^{13}C NMR (151 MHz) (3- β product): 148.0, 139.1, 128.3 (2 C), 127.7 (2 C), 127.3, 120.9, 74.7, 69.8, 56.2 (2 C), 54.5, 42.5, 39.9, 39.5, 37.6, 36.2, 36.0, 35.8, 35.4, 33.1, 32.3, 28.2, 28.0, 25.8, 24.2, 23.8, 22.8, 22.5, 21.0., 18.8, 18.7, 12.0.

3-Benzyloxy-cholestane-3,4-diol (11). Borane-dimethyl sulfide (45 mL of a 2 M solution in THF) was added to compound **10** (8.65 g, 18.1 mmol) at 0 °C under a N_2 atmosphere over 10 min. After 30 min of stirring, the reaction was removed from the ice bath and stirred at room temperature for 3 h before adding 3 mL of 6 M NaOH dropwise. After an additional 15 min, 3 mL of 30% H_2O_2 was added dropwise and the resulting mixture was stirred vigorously at 50 °C for 16 h. The reaction was allowed to cool to room temperature, quenched with water and extracted with 4:1 hexanes/EtOAc. The organic layer was washed with brine and then sodium bicarbonate, dried over Na_2SO_4 and evaporated under reduced pressure. Silica gel chromatography with 19:1 hexanes/ EtOAc yielded a 6.7: 1 mixture of 3 β ,4 α ,5 α /3 α ,4 β ,5 β **11** as a white solid (5.27g, 59% yield). ^1H NMR (600 MHz) (mixture): ^1H NMR (600 MHz) (mixture): 4.703(3 α ,5 β) (1H, d, $J = 11.5$ Hz), 4.699(3 β ,5 α) (1H, d, $J = 11.6$ Hz), 4.476(3 α ,5 β) (1H, d, $J = 11.5$ Hz), 4.476(3 β ,5 α) (1H, d, $J = 11.6$ Hz), 3.864(3 α ,5 β) (1H, m), 3.415(3 β ,5 α) (1H, m), 3.228(3 α ,5 β) (1H, m), 3.180(3 β ,5 α) (1H, m), 2.519(3 α ,5 β) (1H, d, $J = 1.4$ Hz), 2.481(3 β ,5 α) (1H, d, $J = 1.4$ Hz), 0.898(3 β ,5 α) (3H, d, $J = 6.6$ Hz), 0.866(3 β ,5 α) (3H, d, $J = 6.6$ Hz), 0.861(3 β ,5 α) (3H, d, $J = 6.6$ Hz), 0.840(3 β ,5 α) (3H, s), 0.648(3 β ,5 α) (3H, s).

(3 ξ ,5 ξ)-3-Benzoyloxy-cholestan-4-one (12). To a solution of **11** (4.97 g, 10.4 mmol) in 25 mL of DCM was added PCC (10.4 g, 48.4 mmol). The mixture was stirred for 16 h. Hexanes (25 mL) was added to the mixture before filtering it through silica gel. The product was eluted with 4:1 hexanes/EtOAc to yield 71.2 mg of 7: 1 (3 β ,5 α /3 α ,5 β) mixture of **12** as a white solid (4.40 g, 89% yield). ¹H NMR (600 MHz) : 4.861 (1H, d, *J* = 12.1 Hz), 4.846 (1H, d, *J* = 12.0 Hz), 4.490 (1H, d, *J* = 12.0 Hz), 4.464 (1H, d, *J* = 12.0 Hz), 3.937 (1H, dd, *J* = 7.4, 12.0 Hz), 3.828 (1H, dd, *J* = 6.6, 12.3 Hz), 0.899 (3H, d, *J* = 6.5 Hz), 0.866 (3H, d, *J* = 6.6 Hz), 0.862 (3H, d, *J* = 6.6 Hz), 0.747 (3H, s), 0.648 (3H, s), 0.626 (3H, s).

5 α -3 β -Benzoyloxy-cholestan-4-one (12b). To a solution of **12** (4.08 g, 8.30 mmol) in 100 mL of 10% methanolic NaOH was added 50 mL of EtOH. The mixture was heated at reflux for 1 h. The solvent was removed under reduced pressure before extraction of the product with 5% hydrochloric acid and 2:1 hexane/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under reduced pressure to afford **12b** as a white solid (4.01 g, 98% yield). ¹H NMR (600 MHz): 4.845 (1H, d, *J* = 12.0 Hz), 4.464 (1H, d, *J* = 12.0 Hz), 3.936 (1H, dd, *J* = 7.3, 12.1 Hz), 2.252 (1H, m), 2.079 (1H, dd, *J* = 2.8, 12.1 Hz), 0.898 (3H, d, *J* = 6.5 Hz), 0.866 (3H, d, *J* = 6.6 Hz), 0.861 (3H, d, *J* = 6.6 Hz), 0.746 (3H, s), 0.647 (3H, s). ¹³C NMR (151 MHz): 210.5, 138.2, 128.4 (2 C), 127.8 (2 C), 127.6, 81.3 71.8, 57.5, 56.3, 56.2, 54.5, 43.0, 42.6, 39.9, 39.5, 36.4, 36.1, 35.7, 34.9, 30.4, 28.2, 28.0, 24.1, 23.8, 22.849, 22.5, 21.7, 20.3, 18.7, 13.6, 12.0.

3 β -Benzoyloxy-4-methylcholestan-4 β -ol (13). To a mixture of Mg (568.9 mg, 23.5 mmol) and I₂ (28.6 mg, 0.11 mmol) in 5 mL of anhydrous Et₂O was added a solution of MeI (1.1 mL, 17.5 mmol) in 3.9 mL of Et₂O dropwise under a N₂ atmosphere over 20 min. After 15 min, a solution of **12** (815.7 mg, 1.7 mmol) in 60 mL of Et₂O was added dropwise over 30 min. After an additional 5 min, the reaction was cooled on ice before extraction with cold saturated aqueous NH₄Cl and 2:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under reduced pressure. The crude product was purified by flash chromatography with 19:1 hexanes/EtOAc to afford **13** as a white solid

(829.8 g, 98% yield). ^1H NMR (600 MHz): 7.348 (4H, d, $J = 4.3$ Hz), 7.292 (1H, m), 4.701 (1H, d, $J = 11.6$ Hz), 4.500 (1H, d, $J = 11.6$ Hz), 3.038 (1H, dd, $J = 4.6, 11.4$ Hz), 1.219 (3H, s), 1.048 (3H, s), 0.900 (3H, d, $J = 6.6$ Hz), 0.869 (3H, d, $J = 6.6$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.652 (3H, s), 0.555 (1H, m). ^{13}C NMR (151 MHz): 138.7, 128.3 (2C), 127.8 (2C), 127.6, 82.9, 74.1, 71.2, 56.7, 56.3, 55.8, 53.3, 42.6, 40.1, 39.5, 36.7, 36.24, 36.20, 35.8, 35.0, 32.6, 28.3, 28.0, 25.7, 24.2, 23.8, 22.8, 22.7, 22.6, 20.73, 20.70, 18.7, 14.1, 12.1.

4-Methylcholestane-3 β ,4 β -diol (3). To a solution of **13** (779.9 mg, 1.5 mmol) in 15 mL of EtOAc was added 133.3 mg of 10% Pd/C. The mixture was stirred under H_2 for 1.5 h. The crude product was filtered through silica gel to afford **3** (570.8 mg, 89% yield). ^1H NMR (600 MHz): 3.244 (1H, m), 1.224 (3H, s), 1.009 (3H, s), 0.895 (3H, d, $J = 6.6$ Hz), 0.863 (3H, d, $J = 6.6$ Hz), 0.859 (3H, d, $J = 6.6$ Hz), 0.646 (3H, s), 0.581 (1H, m). ^{13}C NMR (151 MHz): 75.7, 74.2, 56.6, 56.3, 55.6, 52.9, 42.5, 40.0, 39.5, 36.6, 36.2, 36.19, 35.8, 34.9, 32.5, 28.3, 28.0, 27.2, 25.4, 24.2, 23.8, 22.8, 22.5, 20.6, 20.59, 18.7, 14.0, 12.1.

(24R)-4-methylsigmast-22-en-3 β ,4 β -diol (6) from stigmasterol. ^1H NMR (600 MHz): 5.144 (1H, m), 5.012 (1H, m), 3.247 (1H, m), 1.226 (3H, s), 1.013 (3H, s), 1.004 (3H, d, $J = 6.6$ Hz), 0.846 (3H, d, $J = 6.4$ Hz), 0.804 (3H, t, $J = 7.4$ Hz), 0.786 (3H, d, $J = 6.4$ Hz), 0.668 (3H, s), 0.590 (1H, m). ^{13}C NMR (151 MHz): 138.4, 129.2, 75.7, 74.2, 56.7, 56.1, 55.7, 52.9, 51.2, 42.4, 40.5, 39.9, 36.6, 36.2, 34.9, 32.5, 31.9, 29.0, 27.2, 25.4, 25.36, 24.2, 21.2, 21.1, 20.6, 20.57, 19.0, 14.0, 12.3, 12.2.

(24R)-4-methylstigmastane-3 β ,4 β -diol (7) from stigmasterol. ^1H NMR (600 MHz): 3.247 (1H, m), 1.227 (3H, s), 1.011 (3H, s), 0.904 (3H, d, $J = 6.5$ Hz), 0.845 (3H, t, $J = 7.4$ Hz), 0.834 (3H, d, $J = 6.8$ Hz), 0.813 (3H, d, $J = 6.8$ Hz), 0.649 (3H, s), 0.584 (1H, m). ^{13}C NMR (151 MHz): 75.7, 74.2, 56.6, 56.2, 55.6, 52.9, 45.9, 42.5, 40.0, 36.6, 36.2, 36.15, 34.9, 34.0, 32.5, 29.2, 28.3, 27.2, 26.1, 25.4, 24.2, 23.1, 20.6, 20.58, 19.8, 19.0, 18.7, 14.0, 12.1, 12.0.

(4R)-Spiro(oxirane-2,4'-5 α -cholestan-3 β -ol (15). To a solution of **14** (100.2 mg, 0.2500 mmol) in 3 mL of DCM was added 70% mCPBA (50.6 mg, 0.26 mmol). The mixture was stirred for 30 min before extraction with saturated aqueous sodium bicarbonate and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and filtered through alumina. The solvent was removed under reduced pressure to afford **15** (93.9 mg, 90% yield).

(4R)-3-N-phenylcarbamate-spiro(oxirane-2,4'-5 α -cholestan-3 β -ol (16). To a solution of **15** (61.2 mg, 0.150 mmol) in 3 mL of anhydrous DCM and 100 μ L of pyridine was added of phenyl isocyanate (100 μ L, 0.92 mmol) under a N₂ atmosphere. The mixture was stirred at room temperature for 16 h before extraction with 10% hydrochloric acid and 2:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under reduced pressure. The crude product was purified by silica gel chromatography with 9:1 hexanes/EtOAc to provide **16** (72.0 mg, 92% yield, 99% yield based on recovered starting material). ¹H NMR (600 MHz): 7.044 (1H, t, *J* = 7.3 Hz), 6.560 (1H, s), 4.986 (1H, dd, *J* = 5.2, 12.1 Hz), 2.911 (1H, d, *J* = 4.4 Hz), 2.652 (1H, d, *J* = 5.0 Hz), 0.907 (3H, d, *J* = 6.6 Hz), 0.870 (3H, d, *J* = 6.6 Hz), 0.868 (3H, s), 0.865 (3H, d, *J* = 6.6 Hz), 0.653 (3H, s). ¹³C NMR (151 MHz): 152.6, 137.8, 129.0, 123.4, 118.8, 72.1, 59.8, 56.3, 56.1, 54.6, 47.8, 45.7, 42.5, 39.9, 39.5, 38.4, 36.5, 36.1, 35.8, 35.3, 31.1, 28.2, 28.0, 27.4, 24.2, 23.8, 22.8, 22.5, 21.2, 19.3, 18.7, 13.6, 12.0.

(4 ξ)-Cholestan-3 β -ol-4-cyclic carbonate (17). A solution of **16** (72.0 mg, 0.13 mmol) in 5 mL of 2% TFA in anhydrous DCM was stirred at room temperature for 2 h. The reaction was quenched with 200 μ L of pyridine and the solvent was evaporated under a flow of N₂. The residue was extracted with 10% hydrochloric acid and 2:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under reduced pressure. The crude product was purified by silica gel chromatography with 9:1-1:1 hexanes/EtOAc to provide a 1/1.5 **17a:17b** mixture (29.2 mg, 42% yield). **(4R)-Cholestan-3 β -ol-4-cyclic carbonate (17a).** ¹H NMR (600 MHz): 4.469 (1H, d, *J* = 8.3 Hz), 4.224 (1H, d, *J* = 8.3 Hz), 3.771 (1H, m), 0.894 (3H, d, *J* = 6.6 Hz), 0.866 (3H, d, *J* = 6.6 Hz), 0.861 (3H, d, *J* = 6.6 Hz), 0.722

(3H, s), 0.642 (3H, s). ^{13}C NMR (151 MHz): 154.8, 88.6, 74.4, 64.8, 56.2, 56.19, 54.9, 50.1, 42.4, 39.7, 39.5, 37.6, 36.1, 35.9, 35.8, 35.1, 31.3, 28.2, 28.0, 26.7, 24.2, 23.8, 22.8, 22.5, 20.8, 20.0, 18.7, 13.3, 12.0.

(4S)-Cholestan-3 β -ol-4-cyclic carbonate (17b). ^1H NMR (600 MHz): 4.469 (1H, d, J = 8.3 Hz), 4.224 (1H, d, J = 8.3 Hz), 3.771 (1H, m), 0.894 (3H, d, J = 6.6 Hz), 0.866 (3H, d, J = 6.6 Hz), 0.861 (3H, d, J = 6.6 Hz), 0.722 (3H, s), 0.642 (3H, s). ^{13}C NMR (151 MHz): 155.1, 86.1, 72.7, 69.8, 56.4, 56.2, 55.1, 51.7, 42.6, 39.8, 39.5, 36.9, 36.16, 36.15, 35.7, 35.0, 31.8, 28.2, 28.0, 27.6, 24.1, 23.8, 22.8, 22.5, 20.8, 20.1, 18.7, 14.2, 12.1.

(4S)- 3 β -Ethoxymethoxy-cholestan-4-cyclic carbonate (18). To a solution of **17b** (5.9 mg, 0.013 mmol) and 150 μL of $i\text{Pr}_2\text{EtN}$ in 0.5 mL of anhydrous THF was added chloromethyl ethyl ether (20 μL , 0.22 mmol). The mixture was heated at reflux under a N_2 atmosphere for 3 h. The reaction was extracted at room temperature with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified via preparative TLC with 4:1 hexanes/EtOAc to give **18** (5.4 mg, 81% yield). ^1H NMR (600 MHz): 4.811 (1H, d, J = 7.3 Hz), 4.680 (1H, d, J = 7.3 Hz), 4.292 (1H, d, J = 8.0 Hz), 4.180 (1H, d, J = 7.9 Hz), 3.686 (1H, m), 3.588 (1H, m), 3.306 (1H, dd, J = 5.2, 11.8 Hz), 1.208 (3H, t, J = 7.1 Hz), 1.030 (3H, s), 0.894 (3H, d, J = 6.5 Hz), 0.863 (3H, d, J = 6.6 Hz), 0.859 (3H, d, J = 6.6 Hz), 0.654 (3H, s), 0.621 (1H, m). ^{13}C NMR (151 MHz): 155.2, 93.4, 85.3, 78.6, 69.9, 64.1, 56.4, 56.2, 55.2, 51.4, 42.6, 39.8, 39.5, 37.0, 36.2, 35.8, 35.7, 35.0, 31.8, 28.2, 28.0, 24.1, 23.8, 23.5, 22.8, 22.5, 20.8, 20.2, 18.6, 15.0, 14.3, 12.1.

(4S)-3 β -Ethoxymethoxy-4-hydroxymethyl-cholestan-4-ol (19). A solution of **18** (5.4 mg, 0.01 mmol) in 1 mL of 10% methanolic NaOH was heated at reflux for 2 h. The solvent was evaporated under a flow of N_2 and the residue was extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to obtain **19** (4.5 mg, 88% yield). ^1H NMR (600 MHz): 4.791 (1H, d, J = 6.6 Hz), 4.703 (1H, d, J = 6.6 Hz), 3.672 (1H, m), 3.617-3.537 (3H, m), 3.493 (1H, d, J = 7.2 Hz), 1.231 (3H, t, J = 7.1 Hz), 1.066 (3H, s), 0.894 (3H, d, J =

6.5 Hz), 0.864 (3H, d, $J = 6.6$ Hz), 0.859 (3H, d, $J = 6.6$ Hz), 0.646 (3H, s), 0.623 (1H, m). ^{13}C NMR (151 MHz): 93.8, 78.2, 75.3, 64.2, 64.0, 56.6, 56.3, 55.6, 47.5, 42.5, 40.0, 39.5, 36.6, 36.2 (2 C), 35.8, 34.9, 32.3, 28.3, 28.0, 24.1, 23.8, 23.0, 22.8, 22.6, 20.7, 20.5, 18.7, 15.0, 14.6, 12.1.

(4S)-3 β -Ethoxymethoxy-4-methylcholestan-4-ol (20). To a solution of **19** (4.5g, 0.009 mmol) and TEA (25 μL , 0.18 mmol) in 1 mL of DCM at 0 $^{\circ}\text{C}$ was added methanesulfonic acid (6 μL , 0.09 mmol). The mixture was stirred for 30 min and extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to obtain the desired mesylate. ^1H NMR (600 MHz): 4.826 (1H, d, $J = 6.9$ Hz), 4.748 (1H, d, $J = 6.9$ Hz), 4.075 (2H, dd, $J = 9.3, 16.4$ Hz), 3.658-3.597 (2H, m), 3.576 (1H, dd, 5.1, 11.3 Hz), 3.006 (3H, s), 1.221 (3H, t, $J = 7.1$ Hz), 1.055 (3H, s), 0.896 (3H, d, $J = 6.5$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.861 (3H, d, $J = 6.9$ Hz), 0.648 (3H, s). In a solution of the mesylate in 1.5 mL of Et_2O was added 6.5 mg of LAH. The mixture was stirred for 45 min and extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified via preparative TLC with 4:1 hexanes/EtOAc to give **20** (2.9 mg, 67% yield from **19**). ^1H NMR (600 MHz): 4.836 (1H, d, $J = 7.0$ Hz), 4.836 (1H, d, $J = 7.0$ Hz), 4.698 (1H, d, $J = 7.0$ Hz), 3.645 (2H, q, $J = 7.1$ Hz), 3.230 (1H, m), 1.212 (3H, t, $J = 7.1$ Hz), 1.194 (3H, s), 1.037 (3H, s), 0.895 (3H, d, $J = 6.5$ Hz), 0.864 (3H, d, $J = 6.6$ Hz), 0.860 (3H, d, $J = 6.6$ Hz), 0.647 (3H, s), 0.561 (1H, m). ^{13}C NMR (151 MHz): 93.8, 81.4, 74.1, 63.5, 56.7, 56.3, 55.7, 53.2, 42.6, 40.1, 39.5, 36.7, 36.2, 35.8, 35.0, 32.6, 29.7, 28.3, 23.5, 22.8, 22.6, 20.72, 20.70, 18.7, 15.1, 14.1, 12.1

4-Methylcholestan-3 β ,4 β -diol (3) from 20. To a solution of **20** (2.9 mg, 0.006 mmol) in 1mL of 4:1 MeOH/ H_2O was added a mixture of 200 μL 2% sulfuric acid in THF. The reaction was heated at reflux for 1 h before extraction at room temperature with saturated sodium bicarbonate and 2:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated under a flow of N_2 . The crude product was purified via preparative TLC with 2:1 hexanes/EtOAc to give **5** (2.3 mg, 90% yield).

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CHAPTER 5

Synthesis of Simple Analogs of Petrosterol and Potential Enzymatic Products

Abstract

The synthesis of two simple analogs of petrosterol and the possible products of enzymatic reactions via invertebrate phytosterol dealkylation are reported. All five reported sterols were generated from a common intermediate 6 β -methoxy-3 α ,5 α -cyclo-24-cyclopropylcholane-24-one. The two simple analogs, (24R)-24-cyclopropyl-24-methylchol-5-en-3 β -ol and (24S)-24-cyclopropyl-24-methylchol-5-en-3 β -ol, were made as a mixture that was difficult to separate by HPLC. The possible enzymatic products, 24-cyclopropyl-24-methylenchol-5-en-3 β -ol and (24R)-24,25-epoxy-24-cyclopropyl-24-homochol-5-en-3 β -ol and (24S)-24,25-epoxy-24-cyclopropyl-24-homochol-5-en-3 β -ol, were obtained directly from the intermediate ketone 24-cyclopropylchol-5-en-3 β -ol-24-one. The epoxides were made as mixture that was inseparable by HPLC with MeOH. All the products including the mixtures were characterized by 600 MHz NMR. Like petrosterol, all the reported products are potential inhibitors of invertebrate sterol metabolism.

5.1 Introduction

Petrosterol (**1**) and its natural analogs are 26,27-cyclopropyl sterols that have been isolated from many marine sponge species (Figure 5.1).¹⁻¹³ Many oxygenated petrosterol analogs exhibit biological

activities that include anticancer and antifouling.⁸⁻¹³ The stereochemistry of the 24-methyl and side chain 25,26-cyclopropane of petrosterol was established via X-ray crystallography of its p-bromobenzoate derivative.^{1b} Marine invertebrates like crustaceans are unable to biosynthesize sterols and depend on their diet as a source of exogenous sterols including cholesterol, an essential component for somatic and reproductive growth.^{14,15} As in insects, 24-alkyl phytosterols are converted to cholesterol in invertebrates via a well-known dealkylation process (Scheme 5.1).^{14,16-18} Like gorgosterol, petrosterol is a potential inhibitor of invertebrate sterol metabolism. The dealkylation of the 24-methyl in both sterols would lead to a highly reactive cyclopropylcarbiny cation capable of disabling the dealkylating enzyme (Scheme 5.2).^{19,20}

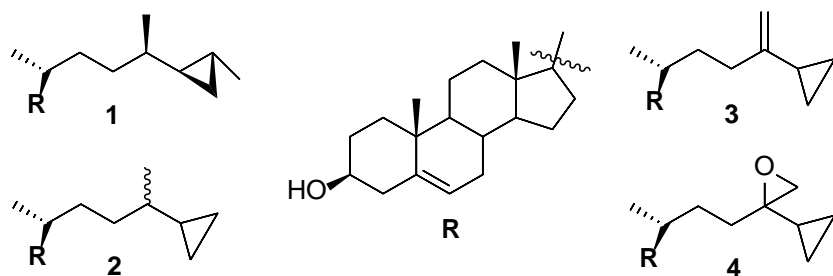
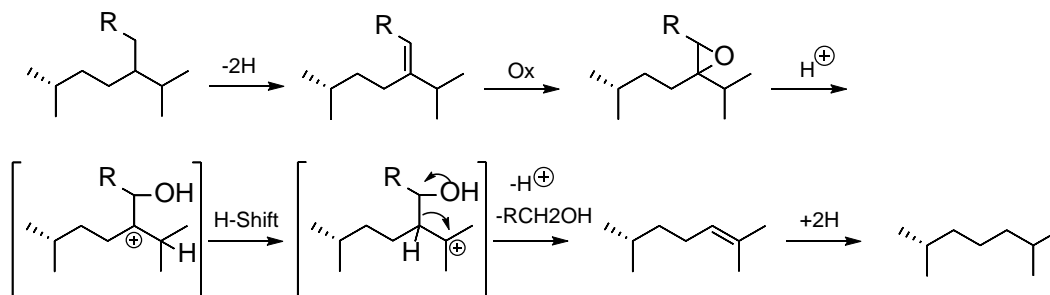
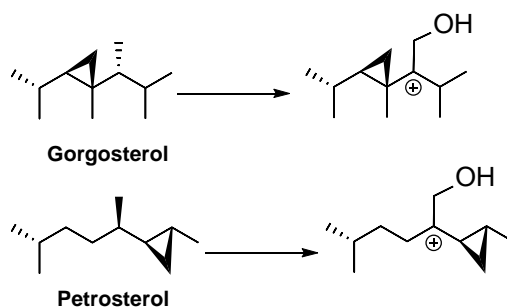


Figure 5.1. Petrosterol **1** and its analogs **2** and their possible metabolic products **3** and **4**.



Scheme 5.1. Mechanism of dealkylation of phytosterols to cholesterol in insects.

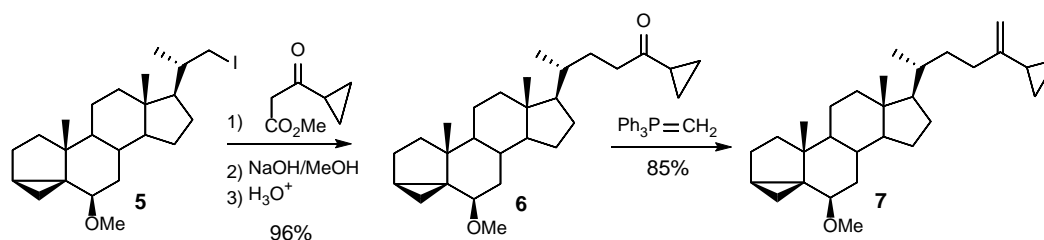


Scheme 5.2. Carbocation intermediates from the dealkylation of gorgosterol and petrosterol.

Two simple analogs of petrosterol, (24R)-24-cyclopropyl-24-methylchol-5-en-3 β -ol and (24S)-24-cyclopropyl-24-methylchol-5-en-3 β -ol (**2**) were synthesized as a mixture as potential inhibitors of invertebrate sterol metabolism. Three possible metabolic products of the analogs **2**, 24-cyclopropyl-24-methylenchol-5-en-3 β -ol **3** and -epoxy-24-cyclopropyl-24-homochol-5-en-3 β -ol (**4**) (as a mixture of isomers), were also synthesized (Figure 5.1).

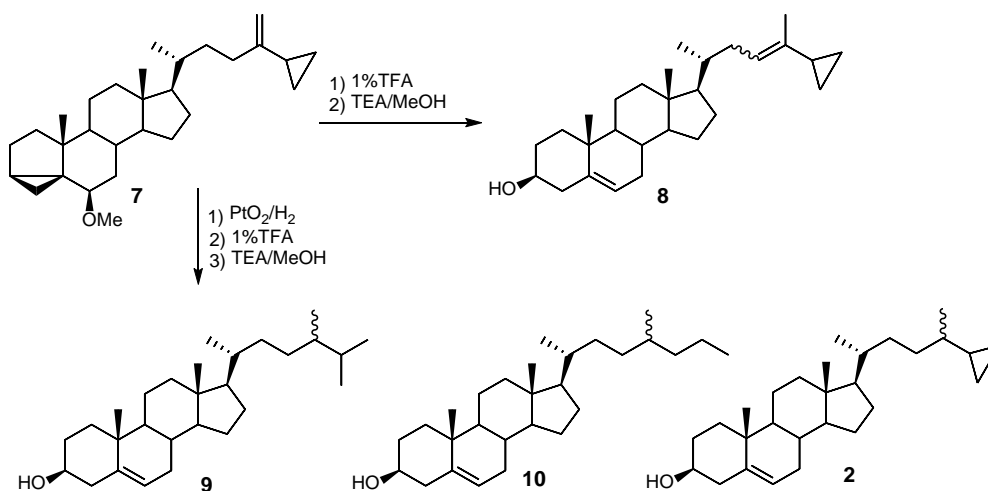
5.2 Results and Discussion

The 6 β -methoxy-3 α ,5 α -cyclo-24-cyclopropylcholane-24-one (**6**), a key intermediate in the synthesis, was made in near quantitative yield via the alkylation of methyl 3-cyclopropyl-3-oxopropionate with *i*-methyl ether 22-iodide followed by saponification and subsequent decarboxylation of the intermediate keto-ester (Scheme 5.3).²¹ A portion of the *i*-methyl ketone **6** was easily converted to intermediate **7** Wittig in a 85% yield. It was hoped that 24-cyclopropyl-24-methylenchol-5-en-3 β -ol **4** could readily be obtained from compound **7** by a simple deprotection of its *i*-methyl ether nucleus. However, the deprotection of intermediate **7** using TFA followed by subsequent transesterification with



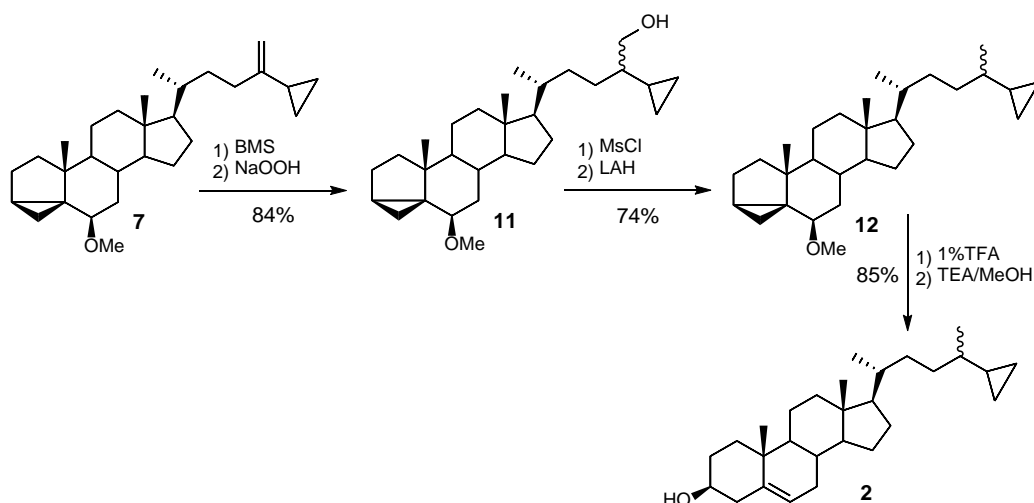
Scheme 5.3. Synthesis of intermediate **7** from i-methyl ether 22-iodide (**5**).

methanolic TEA gave mainly a mixture of the sterols **8a** and **8b** (Scheme 5.4). No stereochemical assignment for the side chain double bond of the sterols **8a** and **8b** was done as those compounds were not of interest. However, sterol **8b** is suspected to be the 23-E isomer as it was the predominant product. Intermediate **7** was hydrogenated in the presence of PtO₂ to reduce the 24-methylene double-bond. Deprotection gave a mixture of the compounds **9**²², **10** and **2** (Scheme 5.4). This method was found to be an inefficient route to synthesize compound **2**, as the desired product yield was unfavorable and the mixture of compounds **9**, **10** and **2** was not easily separable by preparative HPLC. An alternative method to reduce the double bond had to be used because of the sensitivity of the cyclopropane to the hydrogenation reaction.



Scheme 5.4. Synthesis of compounds **8**, **9**, **10** and **2** from intermediate **7**.

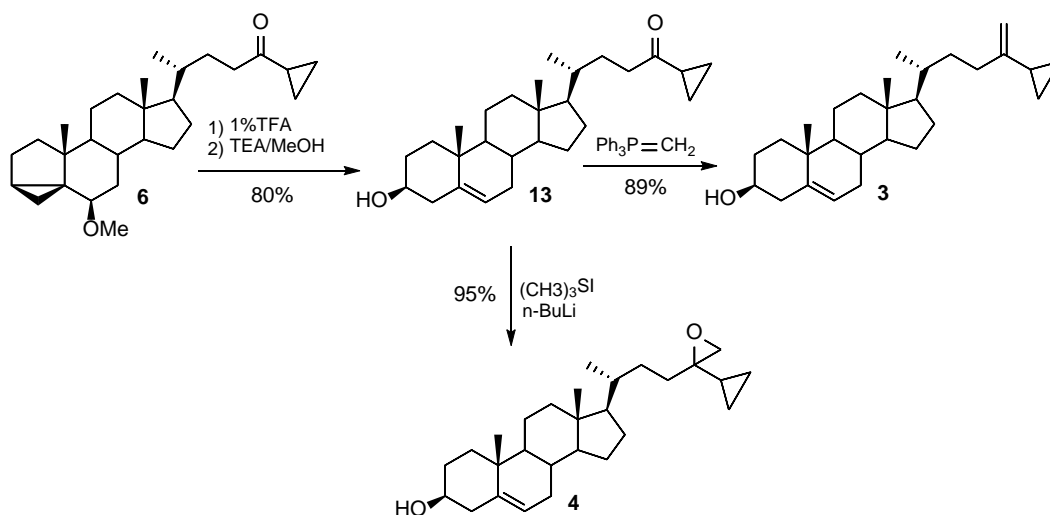
A different approach was used to make compound **2** from intermediate **7** (Scheme 5.5). Intermediate **7** was converted to the mixture of alcohols **11** through a hydroboration-oxidation reaction in an 84% yield.²³ The mixture of alcohols **11** was characterized by NMR and moved forward in the sequence as a mixture, as the alcohols were could not be separated. The mixture of intermediates **11** was deoxygenated in a 2-step procedure to get a compound **12** as a mixture in a 74% yield.²⁴ The i-methyl ethers **12** were deprotected in an 85% yield to afford a mixture of compound **2**, the two simple analogs of petrosterol (24R)-24-cyclopropyl-24-methylchol-5-en-3 β -ol and (24S)-24-cyclopropyl-24-methylchol-5-en-3 β -ol. The isomers could be separated in very small amounts by HPLC after multiple re-injections and were characterized by ¹H NMR. The stereochemical assignment at C24 for the isomers was done chemically by hydrogenating each compound to the corresponding 24-methylcholestane-3 β -ol.²⁵ The ¹³C NMR data for the isomers was obtained as a mixture.



Scheme 5.5. Synthesis of compound **2** from intermediate **7**.

To make compounds **3** and **4** the i-methyl ketone **6** from was deprotected to the corresponding sterol **13** in an 80% yield (Scheme 5.6). From intermediate **13**, compounds **3** and **4** were readily attainable

in just one step. Compound **3** was obtained from intermediate **13** via a Wittig methylenation in an 89% yield. The epoxide mixture (**4**) was made from intermediate **13** using the Corey-Chaykovsky reaction in a 95% yield.²⁶ The mixture of epoxides (**4**) was sensitive to silica gel and was not separable by HPLC with methanol.



Scheme 5.6. Synthesis of compounds **3** and **4** from intermediate **6**.

5.3 Summary

Two simple analogs (**2**) of petrosterol, (24R)-24-cyclopropyl-24-methylchol-5-en-3 β -ol and (24S)-24-cyclopropyl-24-methylchol-5-en-3 β -ol, were successfully synthesized as a mixture from the i-methyl 22-iodide in a six-step sequence with a 43% overall yield. Since the purpose of the sequence was to synthesize those compounds as potential inhibitors of invertebrate sterol metabolism, producing them as a mixture was not an issue. The possible enzymatic products of compound (**2**), 24-cyclopropyl-24-methylenechol-5-en-3 β -ol (**3**) and (24R)-epoxy-24-cyclopropyl-24-hoochol-5-en-3 β -ol and (24S)-24,25-

epoxy-24-cyclopropyl-24-homochol-5-en-3 β -ol (**4**), were also generated in good yield. Biological testing of those compounds has demonstrated their activity as inhibitors of the invertebrate phytosterol dealkylation.

5.4 Experimental

General Procedures. NMR spectra were obtained in CDCl₃ on a Bruker 600 MHz spectrometer (¹H at 600 MHz and ¹³C at 151 MHz) and a Bruker 800 MHz spectrometer (¹H at 800 MHz and ¹³C at 201 MHz). HPLC was performed using a Waters 6000A pump, Waters 410 differential refractometer, and two Altex Ultrasphere ODS columns (5 μ m, 10 x 250 mm) in series at a flow rate of 3 mL/min with MeOH. Preparative thin layer chromatography (TLC) purifications were done with Sorbent Technologies (SORBTECH) silica gel HL thin TLC plates (20 x 20 cm², 250 μ m thick with glass backed support). All solvents were from commercial sources.

6 β -Methoxy-3 α ,5 α -cyclo-24-cyclopropylcholane-24-one (6**).** To a suspension of 63% of NaH (45.2 mg, 1.10 mmol, washed with hexane) in 1 mL of DMF at 0 °C under a N₂ atmosphere was added 300 μ L of methyl 3-cyclopropyl-3-oxopropionate (2.30 mmol) dropwise. The mixture was brought to rt and stirred for 1 h before adding a solution of i-methyl ether 22-iodide (251.2 mg, 0.550 mmol) in 2 mL of DMF. The mixture was stirred at 100 °C for 30 min and cooled to room temperature. The reaction was extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and filtered through silica gel. The solvent was removed under reduced pressure to produce the crude keto-ester as a yellow oil (308 mg). To a solution of the crude product in 1 mL of THF was added 3 mL of 10% methanolic NaOH before heating the mixture at reflux for 30 min. The reaction was cooled before adding 10% hydrochloric acid until aqueous layer was acidic. The mixture was extracted with 4:1

hexanes/EtOAc and the organic layer was washed with brine and dried over Na₂SO₄. The crude product was purified by silica gel chromatography with 39:1-19:1 hexanes/EtOAc to yield 217.1 mg (96% yield). ¹H NMR (600 MHz): 3.315 (3H, s), 2.762 (1H, t, *J* = 2.8 Hz), 2.606-2.533 (1H, m), 2.483-2.413 (1H, m), 1.016 (3H, s), 0.924 (3H, d, *J* = 6.6 Hz), 0.713 (3H, s), 0.640 (1H, m), 0.425 (1H, dd, *J* = 5.1, 8 Hz). ¹³C NMR (151 MHz): 211.7, 82.4, 56.53, 56.51, 56.1, 48.0, 43.4, 42.8, 40.5, 40.3, 35.4, 35.3, 35.0, 33.4, 30.5, 30.0, 28.2, 25.0, 24.2, 22.8, 21.5, 20.3, 19.3, 18.5, 13.1, 12.3, 10.6, 10.5.

6β-Methoxy-3α,5α-cyclo-24-cyclopropyl-24-methylenecholane (7). To a mixture of compound **6** (34.8 mg, 0.084 mmol), methyltriphenylphosphonium iodide (67.3 mg, 0.19 mmol) and KtBuO (18.8 mg, 0.17 mmol) was added 1 mL of anhydrous THF. The mixture was stirred under a N₂ atmosphere at reflux for 1 h. The reaction was allowed to cool to room temperature before extraction with water and 4:1 hexanes/EtOAc. The organic layer was dried over Na₂SO₄ and evaporated under reduce pressure. The crude product was purified via silica gel chromatography with 39:1-19:1 hexanes/EtOAc to afford 29.3 mg of **7** (85% yield). ¹H NMR (600 MHz): 4.603 (1H, m), 4.580 (1H, br), 3.322 (3H, s), 2.767 (1H, t, *J* = 2.8 Hz), 1.024 (3H, s), 0.944 (3H, d, *J* = 6.6 Hz), 0.723 (3H, s), 0.661-0.601 (3H, m), 0.460-0.403 (3H, m). ¹³C NMR (151 MHz): 152.0, 105.5, 82.5, 56.5, 56.2, 48.1, 43.4, 42.8, 40.3, 35.7, 35.3, 35.1, 34.7, 33.4, 32.6, 30.5, 28.3, 25.0, 24.2, 22.8, 21.5, 19.3, 18.6, 16.1, 13.1, 12.3, 6.3, 6.0.

(24ξ)-6β-Methoxy-3α,5α-cyclo-24-cyclopropyl-24-hydroxymethyl-cholane (11). To a solution of **7** (29.3 mg, 0.071 mmol) in 1.5 mL of THF was added 100 μL 2 M BMS dropwise under a N₂ atmosphere. The reaction was stirred for 2 h before adding 10 drops of 10% methanolic NaOH dropwise, followed by 10 drops of 30% H₂O₂. The reaction was stirred at room temperature for 3 h before extracting the product with water and 2:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under a flow of N₂. The crude product was purified by silica gel chromatography with 9:1-4:1 hexanes/EtOAc to obtain 25.5 mg of the mixture **11** (84% yield). The two alcohols in the mixture could

not be separated by TLC or HPLC. ¹H NMR (600 MHz): 3.657-3.573 (4H, m), 3.320 (6H, s), 2.764 (2H, t, *J* = 2.8 Hz), 1.021 (6H, s), 0.929 (6H, d, *J* = 6.6 Hz), 0.925 (6H, d, *J* = 6.6 Hz), 0.715 (6H, s), 0.644 (2H, m), 0.566-0.408 (8H, m), 0.187-0.059 (4H, m). ¹³C NMR (151 MHz): 82.4, 67.3, 66.8, 56.5, 56.2, 56.1, 48.0, 46.5, 46.4, 43.4, 42.8, 40.3, 36.01, 35.98, 35.3, 35.1, 33.4, 33.2, 33.1, 31.9, 29.7, 29.6, 29.3, 28.34, 28.28, 28.1, 25.0, 24.2, 22.8, 22.7, 21.5, 19.3, 18.7, 18.6, 14.1, 13.5, 13.3, 132.3, 3.5, 3.3, 3.2, 2.8.

(24ξ)-6β-Methoxy-3α,5α-cyclo-24-cyclopropyl-24-methylcholane (12). To a solution of **11** (22.2 mg, 0.052 mmol) and TEA (100 μL, 0.72 mmol) of in 1 mL of dry DCM was added MsCl (10 μL, 0.13 mmol) at 0 °C. The mixture was stirred for 30 min at that temperature before sequential extractions with water and 4:1 hexanes/EtOAc. The organic layer was washed with 5% hydrochloric acid followed by a brine wash and dried over Na₂SO₄. The crude product was evaporated under a reduced pressure. A solution of the crude product (28.9 mg) in 1.5 mL of Et₂O was treated with 8.6 mg of LAH. The mixture was stirred for 30 min at room temperature and the reaction was extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The organic layer was washed with brine and dried over Na₂SO₄. The crude product was purified via silica gel chromatography with 39:1-19:1 hexanes/EtOAc to obtain 16.1 mg of the mixture **12** (74% yield). ¹H NMR (600 MHz): 3.323 (6H, s), 2.766 (2H, t, *J* = 2.8 Hz), 1.024 (6H, s), 0.928 (3H, *J* = 6.7 Hz), 0.924 (3H, *J* = 6.7 Hz), 0.920 (3H, *J* = 6.5 Hz), 0.911 (3H, *J* = 6.5 Hz), 0.717 (6H, s), 0.646 (2H, m), 0.587 (2H, m), 0.506-0.305 (8H, m), 0.073 (2H, m), -0.011 (2H, m). ¹³C NMR (151 MHz): 82.5, 56.5, 56.3, 56.2, 48.1, 43.4, 42.8, 40.3, 39.0, 38.9, 36.1, 35.8, 35.3, 35.1, 33.8, 33.5, 33.43, 33.37, 33.3, 31.9, 30.5, 29.7, 29.4, 28.3, 28.2, 25.0, 24.2, 22.8, 22.7, 21.5, 20.1, 19.6, 19.3, 18.8, 18.6, 18.4, 18.0, 14.1, 13.1, 12.3, 4.5, 4.3, 3.1, 2.8.

(24ξ)-24-Cyclopropyl-24-methylchol-5-en-3β-ol (2). To a solution of **12** (34.2 mg, 0.082 mmol) in 1 mL of dry DCM was added 10 μL of TFA. The mixture was stirred for 30 min before adding 1 mL of 10% methanolic TEA. The resulting mixture was heated at 45 °C for 1 h before evaporating the solvent

under a flow of N₂. The observed solid was purified via silica gel chromatography with 19:1-9:1 hexanes/EtOAc to afford 23.3 mg of **2** (71% yield). The mixture was difficult to separate by HPLC with MeOH. It took multiple re-injections to isolate minor amounts of each isomer. **Isomer 2a (24R)-24-Cyclopropyl-24-methylchol-5-en-3β-ol**. ¹H NMR (600 MHz): 5.351 (1H, m), 3.524 (1H, m), 1.010 (3H, s), 0.923 (3H, d, *J* = 6.6 Hz), 0.918 (3H, d, *J* = 6.6 Hz), 0.681 (3H, s), 0.606-0.533 (1H, m), 0.509-0.426 (1H, m), 0.426-0.332 (2H, m), 0.057 (1H, m), -0.003 (1H, m). **Isomer 2b (24ξ)-24-Cyclopropyl-24-methylchol-5-en-3β-ol**. ¹H NMR (600 MHz): 5.352 (1H, s), 3.523 (1H, s), 1.011 (3H, s), 0.927 (3H, d, *J* = 6.6 Hz), 0.926 (3H, d, *J* = 6.6 Hz), 0.682 (3H, s), 0.628-0.560 (1H, m), 0.498-0.431 (1H, m), 0.431-0.374 (1H, m), 0.374-0.307 (1H, m), 0.085 (1H, m), -0.020 (1H, m). **Mixture 2**. ¹³C NMR (151 MHz): 140.8, 121.7, 71.8, 56.80, 56.77, 56.1, 56.0, 50.2, 42.3, 39.8, 39.0, 38.9, 37.3, 36.5, 36.0, 35.8, 33.8, 33.5, 33.4, 33.3, 31.9, 31.7, 28.2, 28.1, 24.3, 21.1, 19.6, 19.4, 18.8, 18.7, 18.4, 18.3, 18.0, 17.6, 11.9, 4.5, 4.3, 3.1, 2.7.

24-Cyclopropyl-chol-5-en-3β-hydroxy-24-one (13). To a solution of **6** (110.3 mg, 0.270 mmol) in 3 mL of dry DCM was added 60 μL of TFA. The mixture was stirred for 30 min before adding 1.5 mL of 10% methanolic TEA. The resulting mixture was heated at 45 °C for 1 h before evaporating the solvent under a flow of N₂. The observed solid was purified via silica gel chromatography with 19:1-9:1 hexanes/EtOAc to afford 85.6 mg of **13** (80% yield). ¹H NMR (600 MHz): 5.345 (1H, m), 3.516 (1H, m), 2.611-2.535 (1H, m), 2.486-2.413 (1H, m), 1.003 (3H, s), 0.929 (3H, d, *J* = 6.6 Hz), 0.677 (3H, s). ¹³C NMR (151 MHz): 211.7, 140.8, 121.6, 71.8, 56.7, 55.9, 50.1, 42.4, 42.3, 40.5, 39.8, 37.3, 36.5, 35.4, 31.90, 31.87, 31.7, 30.0, 28.1, 24.3, 21.1, 20.3, 19.4, 18.5, 11.9, 10.6, 10.5.

24-Cyclopropyl-24-methylenechol-5-en-3β-ol (3). To a mixture of compound **13** (25.5 mg, 0.084 mmol), Ph₃PCH₃I (60.8 mg, 0.17 mmol) and KtBuO (18.8 mg, 0.17 mmol) was added 1 mL of anhydrous THF. The mixture was stirred under a N₂ atmosphere at reflux for 1 h. The reaction was allowed to cool

to room temperature before extraction with water and 4:1 hexanes/EtOAc. The organic layer was dried over Na₂SO₄ and evaporated under reduce pressure. The crude product was purified via silica gel chromatography with 39:1-19:1 hexanes/EtOAc to afford 29.3 mg of **3** (89% yield). ¹H NMR (600 MHz): 5.349 (1H, m), 4.600 (1H, m), 4.578 (1H, br s), 3.520 (1H, m), 1.009 (3H, s), 0.948 (3H, d, *J* = 6.6 Hz), 0.685 (3H, s), 0.652-0.583 (2H, s), 0.458-0.397 (2H, m). ¹³C NMR (151 MHz): 151.9, 140.8, 121.8, 105.5, 71.8, 56.8, 56.0, 50.1, 42.4, 42.3, 39.8, 37.3, 36.5, 35.6, 34.7, 32.6, 31.91, 31.90, 31.7, 28.2, 24.3, 21.1, 19.4, 18.6, 16.1, 11.9, 6.3, 6.0.

(24ξ)-Epoxy-24-cyclopropyl-24-homochol-5-en-3β-ol (4). To a mixture of **13** (19.8 mg, 0.05 mmol) and trimethylsulfonium iodide (36.5 mg, 0.18 mmol) in 1 mL of Et₂O at 0 °C was added 75 μL of 2.5M nBuLi in hexanes. The mixture was stirred for 1 h before quenching the reaction with saturated aqueous Na₂S₂O₃. The mixture was extracted with 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under a flow of N₂. The crude product was purified by flash chromatography with alumina 9:1 hexanes/EtOAc to obtain 20.2 mg of the mixture **4** (99% yield, 96% pure by NMR). The mixture was not separable by HPLC. ¹H NMR (600 MHz): 5.345(2H, m), 3.516 (2H, m), 1.005 (6H, s), 0.927 (6H, d, *J* = 6.6 Hz), 0.681 (6H, s), 0.470-0.398 (2H, m), 0.384-0.278 (4H, m), 0.213-0.136 (2H, m). ¹³C NMR (151 MHz): 140.8, 121.6, 71.8, 59.3, 59.1, 56.8, 56.7, 55.9, 55.8, 51.64, 51.57, 50.1, 42.33, 42.29, 39.8, 37.2, 36.5, 32.7, 32.5, 31.90, 31.88, 31.7, 31.0, 30.9, 28.1, 24.2, 21.1, 19.4, 18.61, 18.59, 13.4, 13.3, 11.9, 2.0, 1.8, 0.63, 0.58.

(23ξ)-24-Cyclopropyl-24-methylchol-5,23-dien-3β-ol (8). The two isomer **8a** and **8b** were isolated by HPLC in a mixture obtained from the PtO₂ catalyzed hydrogenation of compound **7** followed by subsequent deprotection of the i-methyl ethers from the hydrogenation reaction. **Isomer 8a** (minor isomer). ¹H NMR (600 MHz): 5.353 (1H, m), 5.237 (1H, m), 3.524 (1H, m), 1.413 (3H, m), 1.011 (3H, s), 0.946 (3H, d, *J* = 6.6 Hz), 0.694 (3H, s), 0.573 (2H, m), 0.517 (2H, m). **Isomer 8b** (major isomer). ¹H

NMR (600 MHz): 5.352 (3H, m), 5.204 (1H, m), 3.523 (1H, m), 1.483 (3H, br s), 1.009 (3H, s), 0.895 (3H, d, $J = 6.6$ Hz), 0.683 (3H, s), 0.526 (2H, m), 0.416 (2H, m).

(24 ξ)-24-methyl-24-propyl-cholan-3 β -ol (10). The compounds **10** were isolated as a mixture by HPLC in a mixture obtained from the PtO₂ catalyzed hydrogenation of compound **7** followed by subsequent deprotection of the i-methyl ethers from the hydrogenation reaction. ¹H NMR (600 MHz): 0.532 (2H, m), 3.524 (2H, m), 1.010 (6H, s), 0.912 (3H, d, $J = 6.6$ Hz), 0.908 (3H, d, $J = 6.6$ Hz), 0.876 (6H, t, $J = 7.2$ Hz), 0.840 (3H, d, $J = 6.6$ Hz), 0.827 (3H, d, $J = 6.6$ Hz), 0.679 (2H, br s).

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CHAPTER 6

Structural Elucidation of Two Novel 24,25-Cyclopropyl Sterols From a Tropical Jewel Orchid

Abstract:

The structural elucidation by synthesis of two novel cyclopropyl sterols isolated in microgram quantities from a tropical jewel orchid is reported. Since only a minute amount of each sterol was isolated, in order to elucidate their stereochemical configurations, all the synthesized stereoisomers of the candidate structures were characterized by 800 MHz NMR. The determination of the stereochemistry of the side chain cyclopropanes was achieved through a series of correlations ultimately resting on (24R)-24-ethyl-24-methylcholestanol, the structure of which rests on crystallography. These correlations included ones with isotopic labeling of prochiral groups. To our knowledge, this is the first report of sterols containing a side chain cyclopropane in higher plants.

6.1 Introduction

Sterols with cyclopropane-containing side chains are unusual in nature. They have been predominantly observed in marine organisms like dinoflagellates and sea sponges¹. Sterols with nuclear cyclopropanes, like cycloartane triterpenoids, are more common and have been isolated in algae and

higher plants². To date, only three higher plant sterols with cyclopropane side chains have been reported: two cycloartane-type triterpenoids from *Pandanus boninensis*³ and a homocyclotirucallane from *Spiranthes sinensis*⁴. Their structural elucidations were accomplished by spectroscopic methods and X-ray crystallography.

In the present study, two novel sterols from a tropical jewel orchid, 23-[(1*R*,2*S*)-(2-ethyl-1,2-dimethylcyclopropyl)]-24-norchol-5-en-3 β -ol (**1**) and 23-[(1*S*)-(2,2-diethyl-1-methylcyclopropyl)]-24-norchol-5-en-3 β -ol (**2**), are described and their structures were elucidated by 800 MHz ¹H NMR.

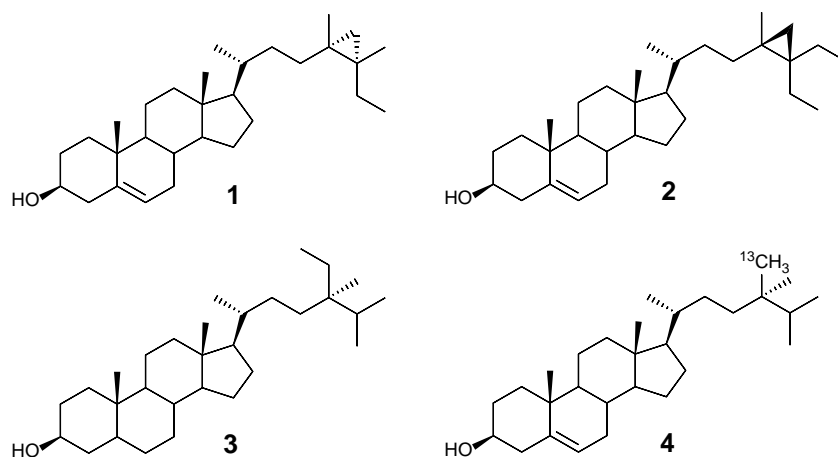


Figure 6.1. Tropical jewel orchid sterols **1** and **2** and the model sterol used in stereochemistry assignments.

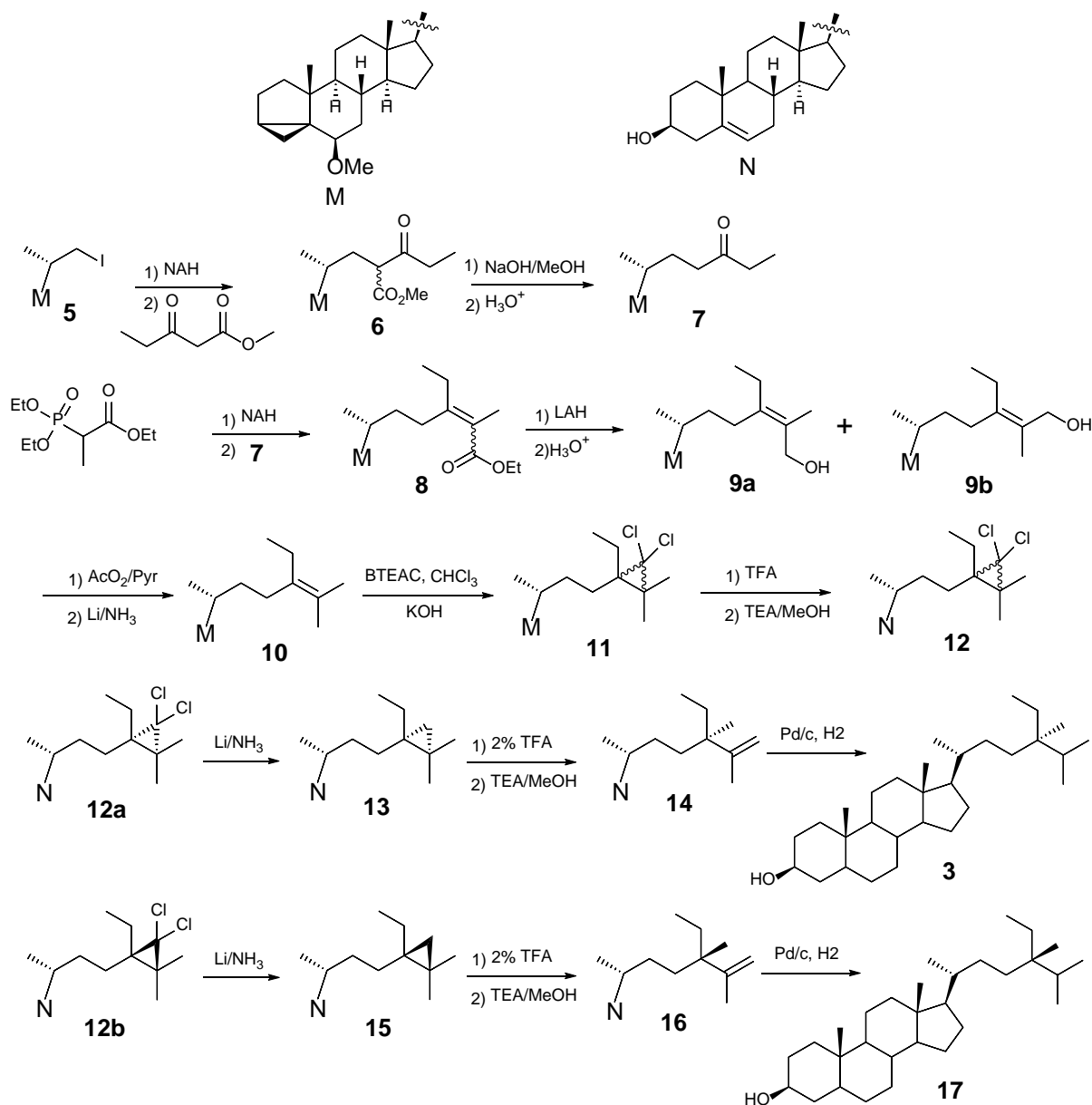
6.2 Results and Discussion

The ^1H NMR of the isolated sterols suggested the presence of a tetrasubstituted cyclopropane in both compounds based on the diagnostic non-equivalent methylene proton signals at δ 0.075 and δ -0.014 for sterol **1** and at δ 0.036 and δ -0.006 for sterol **2**. The possibility of having a nuclear cyclopropane was dismissed for both compounds as they both possess a typical Δ^5 sterol nucleus with the olefinic C6-H δ 5.35 at 3 α -H at δ 3.52. The ^1H NMR spectrum for sterol **1** showed the presence of four methyl singlets, a methyl doublet (d, J = 6.6 Hz) at δ 0.905, and a triplet (t, J = 7.4 Hz) at δ 0.919 indicating an ethyl group. The spectrum of sterol **2** showed three methyl singlets and a methyl doublet (d, J = 6.6 Hz) at δ 0.905 and two triplets (t, J = 7.4 Hz) at δ 0.889 and δ 0.864 for two ethyl groups. The methyl doublet at δ 0.905 (d, J = 6.6 Hz) in both spectra has similar coupling constant to the C21 methyl signal of sterols with the cholesterol side chain. This observation suggested the cyclopropane is likely located toward the end of side chain in both molecules. Therefore, sterols **1** and **2** were suspected to be 24,25-cyclopropyl sterols containing an ethyl and two methyl groups, or two ethyl and one methyl groups, respectively (Figure 6.1).

To confirm the structure and the absolute stereochemistry of each sterol, all the possible isomers of both sterols were synthesized and characterized by 800 MHz ^1H NMR. As a reference, (24*R*)-24-ethyl-24-methylcholestanol (**3**), a known sterol characterized by crystallography⁵, was used to assign the stereochemistry of the 24-ethyl cyclopropanes via a series of correlations. To assign the stereochemistry of the 24-methyl cyclopropyl sterols, isotopic labeling of prochiral groups was used for correlations with the known sterol (24*R*)-24-methyl-28(^{13}C)-codisterol (**4**)⁶.

Because the other sterols in this orchid were mainly 24-ethyl sterols, 24-ethyl-24,25-cyclopropyl sterols intermediates were first synthesized as described in Scheme 6.1. Compound **7**, which contributed the 24-ethyl substituent, was obtained from the 22-*i*-methyl **5** via an acetoacetic ester synthesis to afford **6**, followed by the saponification of the ester and subsequent decarboxylation⁷. The Horner-Wadsworth-Emmons (HWE) reaction was used to obtain a mixture of unsaturated esters **8** from **7**⁶. The selectivity of the HWE reaction was not important in this synthesis, as both *E* and *Z* isomers were desired. The esters were reduced as a mixture with LAH to the allylic alcohols **9a** and **9b**. Those alcohols were separated by silica gel chromatography and the *Z*-alcohol **9a** was the less polar product. The 1,4-reduction product was also observed as a minor product. DIBAL was not a better option for this reduction; it was a much slower reaction and was incompatible with the *i*-methyl ether nucleus. Unequivocal assignment of stereochemistry of the two alcohols (**9a** and **9b**) was based on 2D NMR analyses (COSY and ROESY) were used in the assignment of stereochemistry of the two alcohols. The signals of the methylene protons of the alcohol at δ 4.103 (2H, s) showed a correlation in the ROESY spectrum of **9b** to a multiplet at δ 2.082 (3H, m) that includes the methylene protons of the 24-ethyl substituent. The methylene protons of the ethyl group were determined through COSY correlation to the triplet (3H, t, 7.4 Hz) at δ 0.970. A sample of the mixture was acetylated and deoxygenated via a dissolving metal reduction (Li/NH₃) to obtain the unsaturated *i*-methyl ether **10**⁶. The cyclopropane moiety was introduced as a dichlorocyclopropane via a dichlorocarbene reaction to get a nearly 1:1 mixture of compound **11**⁸. To render the mixture separable by HPLC, the *i*-methyl ethers were deprotected to obtain the respective normal sterol nucleus **12**. After HPLC separation of the mixture, the first sterol to elute was put in the freezer and each sterol (**12a** and **12b**) was handled individually and carefully through the rest of the synthetic sequence to avoid accidental mix-ups. The HPLC peaks were cut in 3 fractions as sterols **12a** and **12b** have similar retention times. The β -cyclopropane **12a** eluted first and was obtained purely in the first fraction of the peak, **12b** was pure in the third fraction. As described in Scheme 6.1, the dichlorocyclopropanes were reduced individually with a dissolving metal reduction (Li/NH₃)⁵ to afford

the ethyl-dimethyl-cyclopropane sterols **13** and **15**. The dechlorinated cyclopropane sterols showed a relatively better separation by HPLC than their precursors and the order of elution was the same. The cyclopropanes were opened via an overnight reaction with TFA. The crude reactions were treated with methanolic TEA to recover any TFA ester sterols generated. The obtained unsaturated sterols **14** and **16** were hydrogenated to form (24*R*)-24-ethyl-24-methylcholestanol (**3**) and its 24-epimer (**17**), respectively. The stereochemistry of **3** at C24, which was established via correlation with a dichlorocyclopropane with X-ray crystallography⁵, permitted the characterization of cyclopropanes **13** and **12a** as β -cyclopropanes. The stereochemistry at C24 for compounds **12b**, **14**, **15**, **16** and **17** was also assigned based on reference compound **3**. Unexpectedly, the NMR spectra of compounds **13** and **15** did not match the isolated sterol that has an ethyl and two methyl groups attached to the cyclopropane. That meant that there was a methyl group at C24 and that the ethyl group and the other methyl group were attached to the same center of the cyclopropane ring.

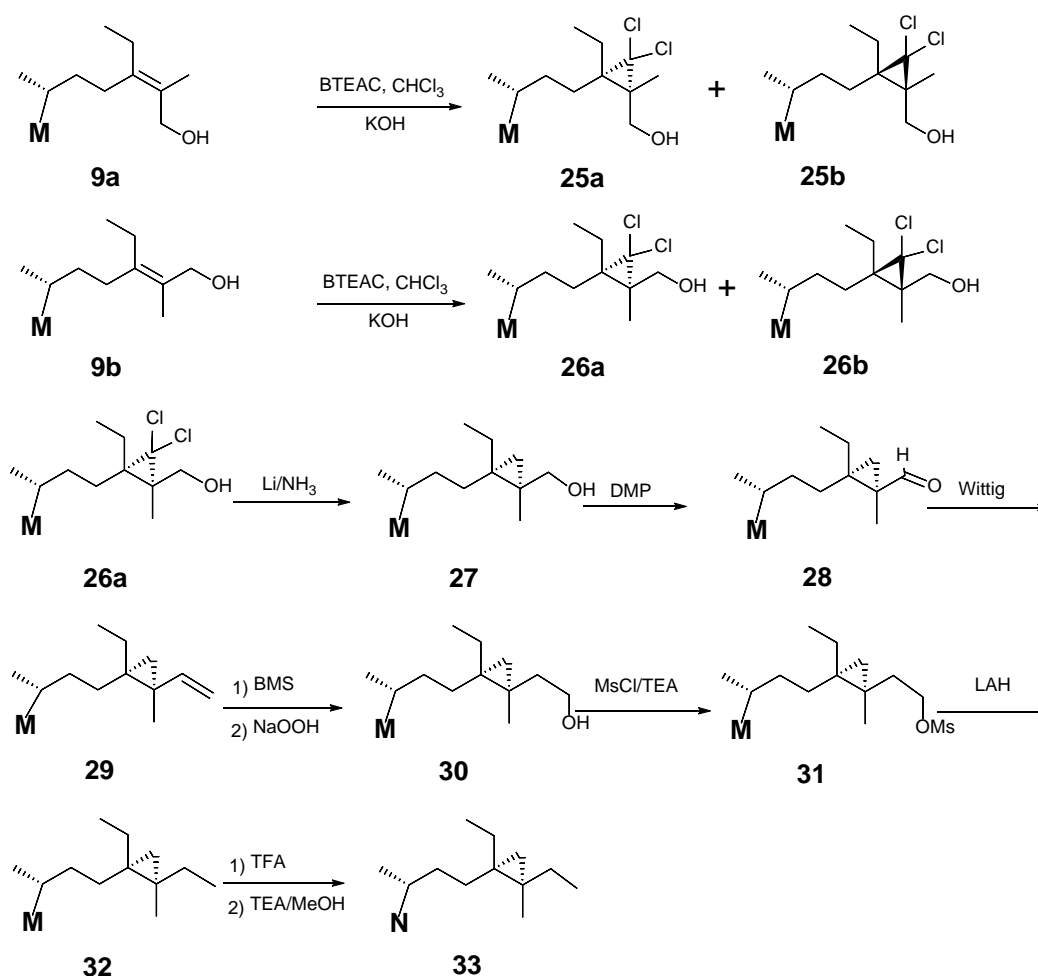


Scheme 6.1. Synthesis of compounds **3** and **17**.

Candidate structures for the orchid cyclopropyl sterol substituted with two ethyl and one methyl groups were synthesized from the intermediate allylic alcohols **9a** and **9b** (Scheme 6.2). The allylic alcohols were dichlorocyclopropanated individually and the product mixtures were separated by HPLC at separate times to avoid any accidental mix-ups. A much better separation was observed for the

dichlorocyclopropyl i-methyl alcohols compared to the normal sterols in Scheme 5.1. Again, the β -cyclopropanes eluted first. The stereochemistry of those cyclopropanes was assigned by chemical correlation of alcohols **25b** and **26b** with compound **15** (Scheme 6.3). Partial reduction of the dichlorocyclopropanes was observed during the LAH reductions. That was not considered an issue as the dichlorocyclopropanes were ultimately reduced to achieve the reference structure **15**.

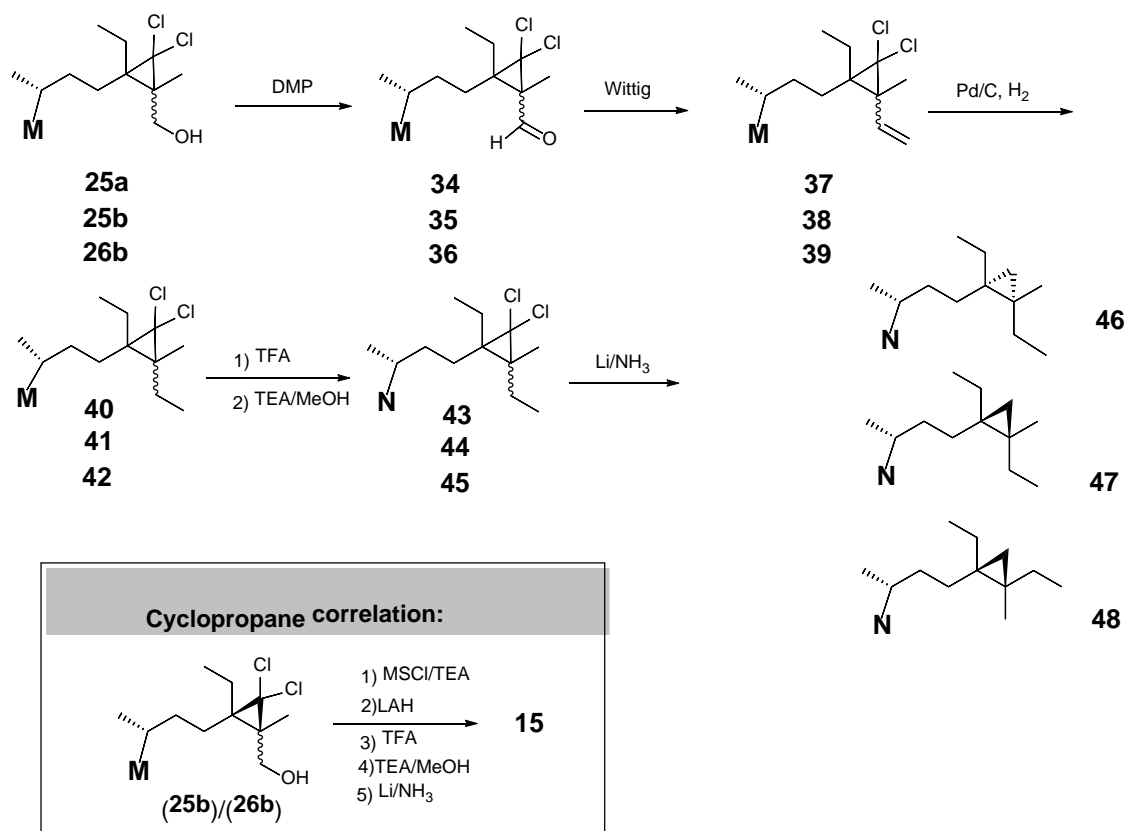
To generate the first 24-diethyl-25methyl-cyclopropyl sterol **33**, the dichlorocyclopropyl alcohol **26a** was first dechlorinated by dissolving metal reduction (Li/NH_3) to get compound **27** (Scheme 6.2). That alcohol was then converted to the unsaturated cyclopropyl i-methyl sterol **29** through a DMP oxidation and subsequent Wittig reaction at room temperature. Due to the observed sensitivity of the cyclopropane to catalytic hydrogenation with Pd/C and PtO_2 ⁵, a different approach was needed to convert compound **29** to the di-ethyl intermediate **32**. Compound **29** was successfully reduced using a selective hydroboration and subsequent two-step deoxygenation⁹. After deprotection of the i-methyl ether of intermediate **32**, compound **33** was obtained. The ^1H NMR spectrum did not match that of the diethyl methyl cyclopropyl sterol.



Scheme 6.2. Synthesis of compounds **33**.

The remaining diastereomers of compound **33** were generated by a different and shorter method (Scheme 6.3). It was found that catalytic hydrogenation could be used if the chlorines were carried through the rest of sequence as a protecting group for the acid-labile cyclopropanes. Thus, compounds **37**, **38** and **39** were hydrogenated to the di-ethyl dichlorocyclopropanes **40**, **41** and **42** which generated the sterols **43**, **44** and **45** after deprotection of the nucleus. The 24,25-diethyl-cyclopropyl sterols **46**, **47** and **48** were obtained after dichlorination of the cyclopropanes. Again, their ^1H NMR spectra did not match that of the natural diethyl-methyl-cyclopropyl sterol. Since none of the synthesized 24,25-diethyl-

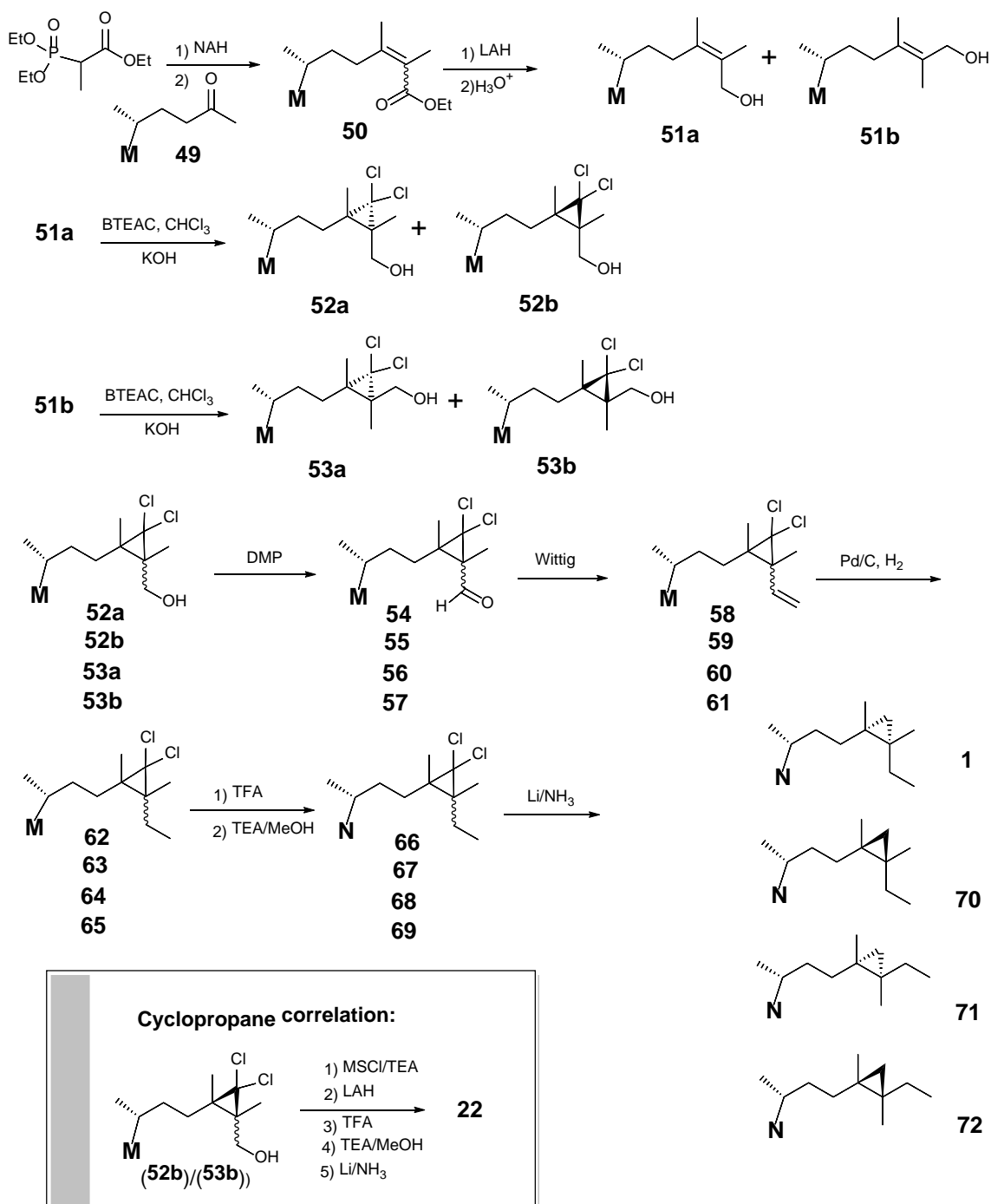
cyclopropyl sterols matched the isolated tropical orchid sterol **2**, it was reasoned that sterol **2** was a 25,25-diethyl-24-methyl-cyclopropyl sterol.



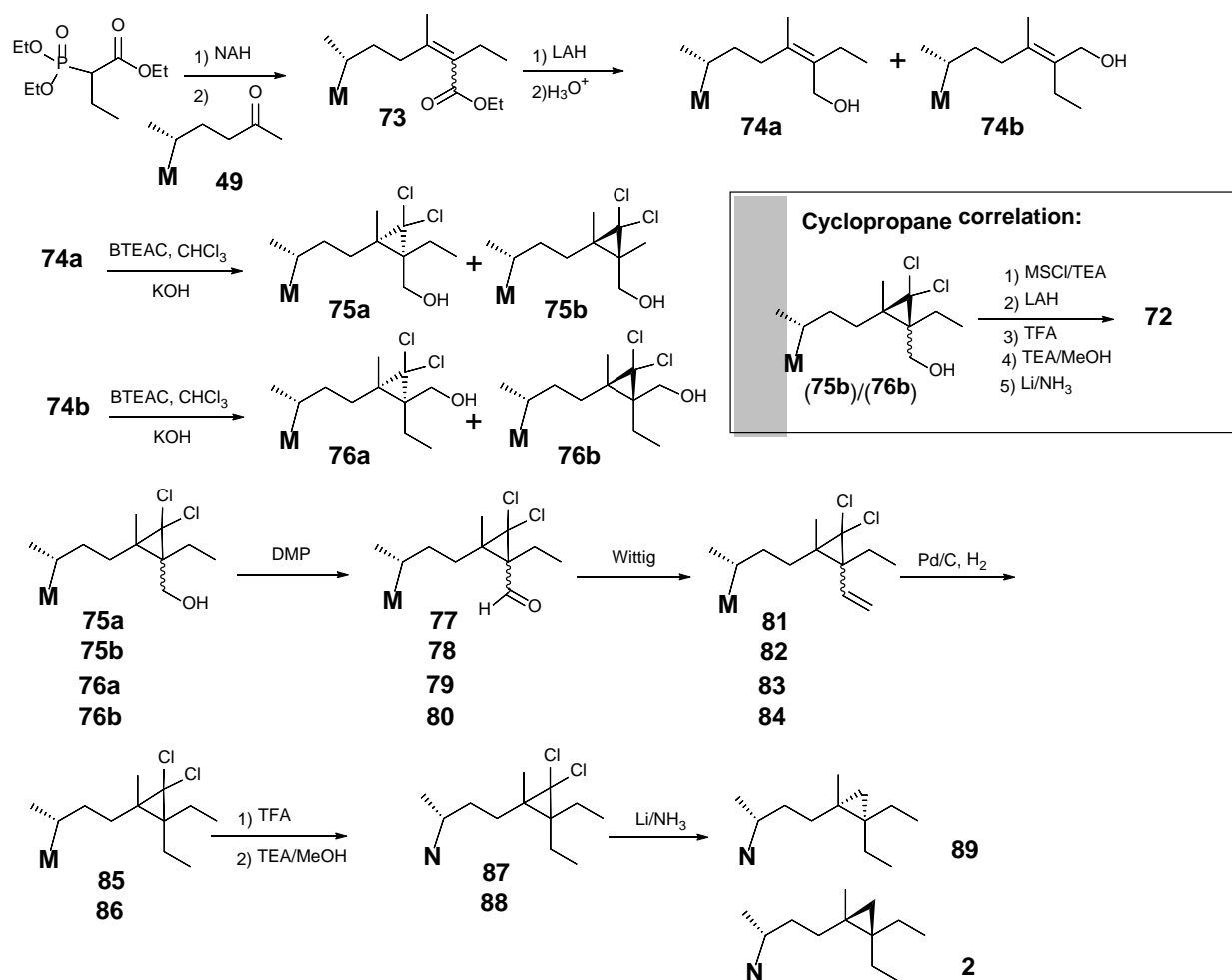
Scheme 6.3. Synthesis of compounds **46**, **47** and **48**. Correlation by synthesis alcohols **27b** and **28b** to compound **15**.

In order to apply a similar logic to the synthesis of 24-methyl-cyclopropyl sterols, stereochemically defined references were needed. Trimethyl-cyclopropyl sterols **21** and **22** were synthesized in scheme similar to the one described in scheme 5.1 (Scheme 6.4). The final stereochemical assignments in this case rests on compound **4**, (24*R*)-24-methyl-28(¹³C)-codisterol, which was characterized by NMR after preparation via a stereospecific enzymatic methylation of 24(¹³C)-methyl-desmosterol with SAM (*S*-adenosylmethionine)⁶. The stereochemistry of the cyclopropanes in **21**

and **22** was the key to the stereochemical assignments of all the 24-methyl-cyclopropyls sterols generated. Just like in Scheme 6.1, the cyclopropanes were introduced to compound **18** using dichlorocarbene to obtain the mixture of i-methyl sterols **19**. After the deprotection the i-methyl ethers, the sterols **20a** and **20b** were separated by HPLC before the reduction of the dichlorocyclopropanes. The β -cyclopropane **20a** eluted earlier, much like the 24-ethyl- β -dichlorocyclopropane **12b**. Deuterated TFA was used in the cyclopropane ring opening reaction of compounds **21** and **22** to add chirality at C24 and allow the differentiation between the two geminal methyls in the products. Even though neat deuterated TFA and dry CDCl_3 were used in the cyclopropane ring opening reactions, the sterols **23** and **24** were only observed as the minor products in a mixture of deuterated and non-deuterated products due to a kinetic isotope effect. Since the experiments were done on a microscale, it is possible that moisture affected the amount of deuterium available for the desired reactions to proceed, as water would promote proton-deuterium exchange before the ring opening reaction could take place. The assignment of α and β methyls in both products **23** and **24** were based on the deuterium shifts observed in the ^{13}C NMR spectra. HMBC and HSQC analyses were used to assign the side chain carbons in the unlabeled 24-dimethyl sterol. C23 at δ 36.8, C24 at δ 38.0 and 24 α -methyl at δ 27.5 in compound **23** were identified based on their deuterium shifts compared to the unlabeled product. Shifts of about 20 ppb were observed for C23 and the unlabeled 24-methyl and a shift of 70 ppb for C24 in compounds **23** and **24**. Signals for the deuterated 24-methyl of **23** and **24** had $\Delta\delta$ values of 320 ppb (t, $J = 19.2$ Hz) and 318 ppb (t, $J = 19.5$ Hz) shifts to the right in their respective ^{13}C -NMR spectrum. The final stereochemical assignments for compounds **24**, **22** and the other sterol in this sequence were assigned based on the monodeuterated methyl (24 α) in **24** matching the ^{13}C methyl (24 α) in the model compound **4** at δ 27.5.



Scheme 6.5. Synthesis of compounds **1**, **70**, **71** and **72**. Correlation by synthesis alcohols **52b** and **53b** to compound **22**.



Scheme 6.6. Synthesis of compounds **2** and **89**. Correlation by synthesis alcohols **75b** and **76b** to compound **72**.

6.3 Conclusions

The two unusual cyclopropyl sterols from the tropical jewel orchid were identified as 23-[(1*R*,2*S*)-(2-ethyl-1,2-dimethylcyclopropyl)]-24-norchol-5-en-3 β -ol (**1**) and 23-[(1*S*)-(2,2-diethyl-1-methylcyclopropyl)]-24-norchol-5-en-3 β -ol (**2**). Both unusual phytosterols and all their possible isomers were synthesized and characterized by 800 MHz ^1H NMR as only minute amounts of both sterols were isolated. Access to an 800 MHz NMR was instrumental to the process of discovering and ultimately characterizing those sterols. To date, this is the first report of the isolation and characterization of sterols with a side chain cyclopropane in higher plants. The presence of those unusual sterols in the aerial parts of those plants could be as chemical defense against herbivorous insects. Those complex sterols may impede the dealkylation process used by insects to convert phytosterols to cholesterol, an essential component for growth and reproduction.^{10,11} Feeding experiments with those sterols would be necessary to verify this hypothesis.

6.4 Experimental

General Procedures. NMR spectra were obtained in CDCl_3 on a Bruker 600 MHz spectrometer (^1H at 600 MHz and ^{13}C at 151 MHz) and a Bruker 800 MHz spectrometer (^1H at 800 MHz and ^{13}C at 201 MHz). HPLC was performed using a Waters 6000A pump, Waters 410 differential refractometer, and two Altex Ultrasphere ODS columns (5 μm , 10 x 250 mm) in series at a flow rate of 3 mL/min with MeOH. Preparative thin layer chromatography (TLC) purifications were done with Sorbent Technologies

(SORBTECH) silica gel HL thin TLC plates (20 x 20 cm², 250 μm thick with glass backed support). All solvents were from commercial sources.

6β-Methoxy-3α,5α-cyclo-27-norcholestan-24-one (7). To a suspension of 63% NaH (64.3 mg, 1.60 mmol) in 1 mL of DMF at 0 °C under a N₂ atmosphere was added 220 μL of methyl propionylacetate (1.76 mmol) dropwise. The mixture was brought to rt and stirred for 1h before adding a solution of i-methyl ether 22-iodide (344.8 mg, 0.760 mmol) in 2 mL of DMF. The mixture was stirred at 100 °C for 30 min and cooled to room temperature. The reaction was extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and filtered through silica gel. The solvent was removed under reduced pressure to afford a crude mixture of **6β-Methoxy-3α,5α-cyclo-23(ξ)-carboxymethyl-27-norcholestan-24-one (6)** (563.9mg). ¹H NMR (600 MHz): 3.727 (3H, s), 3.698 (3H, s) 3.572 (1H, dd, *J* = 4.4, 9.9 Hz), 3.551 (1H, dd, *J* = 3.1, 11.2 Hz), 3.314 (6H, s), 2.761 (2H, t, *J* = 2.7 Hz), 1.070 (3H, t, *J* = 7.3 Hz), 1.053 (3H, t, *J* = 7.2 Hz), 1.011 (3H, s), 1.008 (3H, s), 0.911 (3H, d, *J* = 6.4 Hz), 0.897 (3H, d, *J* = 6.6 Hz), 0.637 (2H, dd, *J* = 4.0, 4.8 Hz), 0.425 (2H, dd, *J* = 5.0, 7.9 Hz). The crude product (**6**) was treated with 3 mL of 10% methanolic NaOH and stirred at reflux for 30 min. The reaction was cooled before adding 10% hydrochloric acid until aqueous layer was acidic. The mixture was extracted with 4:1 hexanes/EtOAc and the organic layer was washed with brine and dried over Na₂SO₄. The crude product (**7**) was purified by silica gel chromatography with 19:1-9:1 hexanes/EtOAc to yield 218.9 mg (72% for the two steps). ¹H NMR (600 MHz): 3.318 (3H, s), 2.764 (1H, t, *J* = 2.8 Hz), 2.422 (3H, m), 2.311 (1H, m), 1.050 (3H, t, *J* = 7.4 Hz), 1.018 (3H, s), 0.905 (3H, d, *J* = 6.5 Hz), 0.710 (3H, s), 0.643 (1H, dd, *J* = 4.0, 4.9 Hz), 0.428 (1H, dd, *J* = 5.0, 8.0 Hz). ¹³C NMR (151 MHz): 212.3, 82.4, 56.5(4), 56.5(1), 56.1, 48.0, 43.4, 42.8, 40.3, 39.4, 35.8, 35.4, 35.3, 35.0, 33.4, 30.5, 30.0, 28.2, 25.0, 24.1, 22.8, 21.5, 19.3, 18.5, 13.1, 12.3, 7.9.

6 β -Methoxy-3 α ,5-cyclo-26,27-bisnorcholestan-24-one (49). ¹H NMR (600 MHz): 3.322 (3H, s), 2.767 (1H, t, *J* = 2.7 Hz), 2.453 (1H, m), 2.334 (1H, m), 1.022 (3H, s), 0.910 (3H, d, *J* = 6.6 Hz), 0.715 (3H, s), 0.647 (1H, dd, *J* = 4.1, 5.0 Hz), 0.432 (1H, dd, *J* = 5.0, 8.0 Hz).

General method for the Horner-Emmons-Wadsworth (HEW) reaction. To a suspension of NaH (2.4 eq.) in 1 mL of dry THF in a stretched test tube was added dropwise the phosphonate ester (2.4 eq.) at 0 °C. The mixture was stirred for 1h under a N₂ atmosphere at rt before adding dropwise a solution of the keto-i-methyl ether (90.3-202.0 mg) in 1mL of THF. The tube was carefully flame sealed before heating the reaction at 85 °C for 18 h. The reaction was allowed to cool to room temperature before extraction with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The pooled organic layers were washed with a brine and dried over Na₂SO₄. The crude product was concentrated under reduced pressure and purified via silica gel chromatography with 39:1-19:1 hexanes/EtOAc to obtain a mixture of the desired products and starting material.

(24 ξ)-Ethyl-6 β -Methoxy-3 α ,5 α -cyclo-stigmast-24-en-26-oate (8). This product was generated via the HEW reaction of triethyl 2-phosphonopropionate with 6 β -Methoxy-3 α ,5 α -cyclo-27-norcholestan-24-one (43% yield). ¹H NMR (600 MHz): 4.179 (4 H, m), 3.325 (3H, s), 3.322 (3H, s), 2.770 (1H, t, *J* = 2.7 Hz), 2.766 (1H, t, *J* = 2.7 Hz), 1.846 (3H, s), 1.840 (3H, s), 1.297 (3H, t, *J* = 7.2 Hz), 1.293 (3H, t, *J* = 7.2 Hz), 1.024 (3H, s), 1.022 (3H, s), 0.985 (3H, d, *J* = 6.6 Hz), 0.957 (3H, d, *J* = 6.6 Hz), 0.728 (3H, s), 0.720 (3H, s), 0.647 (2H, m), 0.431 (2H, m).

(24 ξ)-Ethyl-6 β -Methoxy-3 α ,5 α -cyclo-ergost-24-en-26-oate (50). This product was generated via the HEW reaction of triethyl 2-phosphonopropionate with 6 β -Methoxy-3 α ,5 α -cyclo-26,27-dinorcholestan-24-one (33% yield). ¹H NMR (600 MHz): 4.180 (4 H, m), 3.323 (3H, s), 3.320 (3H, s), 2.769 (1H, t, *J* = 2.6 Hz), 2.765 (1H, t, *J* = 2.7 Hz), 1.969 (3H, br s), 1.849 (3H, br s), 1.831 (3H, br s), 1.754 (3H, br

s), 1.295 (3H, t, $J = 7.2$ Hz), 1.293 (3H, t, $J = 7.2$ Hz), 1.023 (3H, s), 1.020 (3H, s), 0.975 (3H, d, $J = 6.6$ Hz), 0.949 (3H, d, $J = 6.6$ Hz), 0.724 (3H, s), 0.717 (3H, s), 0.645 (2H, m), 0.429 (2H, m).

(24 ξ)-Ethyl-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergost-24-en-26-oate (73). This product was generated via the HEW reaction of triethyl 2-phosphonobutyrate with **49** (21%). The reaction was heated at 110 °C for 92 h. ^1H NMR (600 MHz): 4.194 (4 H, m), 3.326 (3H, s), 3.322 (3H, s), 2.771 (1H, t, $J = 2.7$ Hz), 2.766 (1H, t, $J = 2.7$ Hz), 1.910 (3H, s), 1.756 (3H, s), 1.300 (6 H, t, $J = 7.2$ Hz), 1.026 (3H, s), 1.022 (3H, s), 0.999 (3H, t, $J = 7.4$ Hz), 0.993 (3H, t, $J = 7.4$ Hz), 0.976 (3H, d, $J = 6.6$ Hz), 0.941 (3H, d, $J = 6.6$ Hz), 0.728 (3H, s), 0.717 (3H, s), 0.649 (2H, m), 0.432 (2H, m).

General method for the LAH reduction of the unsaturated i-methyl ether esters. The starting esters (less than 23 mg) were separately dissolved in 1 mL of anhydrous Et_2O . An excess of LAH was added to each reaction in 1 mL of anhydrous Et_2O . After 1 h of stirring, the reactions were worked up with 5 mL of 10% of hydrochloric acid and extracted with 2:1 hexanes/ EtOAc . The organic layers were washed with brine, dried over Na_2SO_4 and filtered through a small pad of silica gel before evaporating the solvents. Crude products were purified on a small scale via preparative TLC with 9:1 hexanes/ EtOAc . The Z-alcohols were less polar than their E-isomers for all the products.

(24Z)-6 β -Methoxy-3 α ,5 α -cyclo-stigmast-24-en-26-ol (9a). ^1H NMR (600 MHz): 4.093 (2H, s), 3.323 (3H, s), 2.767 (1H, t, $J = 2.7$ Hz), 1.742 (3H, s), 1.023 (3H, s), 0.970 (3H, t, $J = 7.4$ Hz), 0.964 (3H, d, $J = 6.6$ Hz), 0.717 (3H, s), 0.647 (1H, dd, $J = 4.0, 4.8$ Hz), 0.430 (1H, dd, $J = 5.1, 8.0$ Hz). ^{13}C NMR (151 MHz): 140.2, 127.1, 82.4, 63.8, 56.6, 56.5, 55.9, 48.0, 43.4, 42.8, 40.3, 36.3, 36.2, 35.3, 35.1, 33.4, 30.5, 28.3, 28.2, 25.7, 25.0, 24.2, 22.8, 21.5, 19.3, 18.7, 15.8, 13.1, 12.8, 12.2.

(24E)-6 β -Methoxy-3 α ,5 α -cyclo-stigmast-24-en-26-ol (9b). ^1H NMR (600 MHz): 4.103 (2H, s), 3.325 (3H, s), 2.769 (1H, t, $J = 2.7$ Hz), 1.738 (3H, s), 1.025 (3H, s), 0.976 (3H, d, $J = 6.6$ Hz), 0.970 (3H, t, $J =$

7.4 Hz), 0.725 (3H, s), 0.648 (1H, dd, $J = 4.0, 4.9$ Hz), 0.432 (1H, dd, $J = 5.1, 8.0$ Hz). ^{13}C NMR (151 MHz): 140.2, 127.1, 82.4, 63.8, 56.6, 56.5, 55.9, 48.0, 43.4, 42.8, 40.3, 36.3, 36.2, 35.3, 35.1, 33.4, 30.5, 28.3, 28.2, 25.7, 25.0, 24.2, 22.8, 21.5, 19.3, 18.7, 15.8, 13.1, 12.8, 12.2.

(24Z)-6 β -Methoxy-3 α ,5 α -cyclo-ergost-24-en-26-ol (51a). ^1H NMR (600 MHz): 4.016 (2H, s), 3.323 (3H, s), 2.767 (1H, t, $J = 2.7$ Hz), 1.737 (3H, s), 1.668 (3H, s), 1.022 (3H, s), 0.957 (3H, d, $J = 6.6$ Hz), 0.715 (3H, s), 0.646 (1H, dd, $J = 4.0, 5.0$ Hz), 0.430 (1H, dd, $J = 5.0, 7.9$ Hz).

(24E)-6 β -Methoxy-3 α ,5 α -cyclo-ergost-24-en-26-ol (51b). ^1H NMR (600 MHz): 4.114 (2H, s), 3.323 (3H, s), 2.768 (1H, t, $J = 2.7$ Hz), 1.745 (3H, s), 1.714 (3H, s), 1.023 (3H, s), 0.967 (3H, d, $J = 6.6$ Hz), 0.722 (3H, s), 0.646 (1H, dd, $J = 4.0, 5.1$ Hz), 0.431 (1H, dd, $J = 5.1, 7.9$ Hz).

(24Z)-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergost-24-en-26-ol (74a). ^1H NMR (800 MHz): 4.119 (2H, m), 3.324 (3H, s), 2.768 (1H, t, $J = 2.8$ Hz), 1.685 (3H, s), 1.025 (3H, s), 0.985 (3H, t, $J = 7.5$ Hz), 0.964 (3H, d, $J = 6.6$ Hz), 0.717 (3H, s), 0.648 (1H, dd, $J = 4.0, 5.0$ Hz), 0.432 (1H, dd, $J = 5.1, 8.0$ Hz). ^{13}C NMR (201 MHz): 134.6, 133.9, 82.5, 61.7, 56.5, 55.9, 48.0, 43.4, 42.8, 40.3, 36.0, 35.5, 35.3, 35.1, 33.4, 30.8, 30.5, 28.3, 25.0, 24.2, 23.8, 22.8, 21.5, 19.3, 18.8, 18.3, 13.3, 13.1, 12.2.

(24E)-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergost-24-en-26-ol (74b). ^1H NMR (800 MHz): 4.133 (2H, s), 3.327 (3H, s), 2.770 (1H, t, $J = 2.6$ Hz), 1.730 (3H, s), 1.027 (3H, s), 1.005 (3H, t, $J = 7.5$ Hz), 0.972 (3H, d, $J = 6.6$ Hz), 0.728 (3H, s), 0.650 (1H, dd, $J = 4.1, 5.0$ Hz), 0.434 (1H, dd, $J = 5.1, 7.9$ Hz). ^{13}C NMR (201 MHz): 134.5, 133.8, 82.5, 62.1, 56.6, 56.5, 48.1, 43.4, 42.8, 40.3, 36.2, 35.3, 35.1, 34.8, 33.4, 31.0, 30.5, 28.3, 25.0, 24.2, 23.5, 22.8, 21.5, 19.3, 18.8, 18.0, 13.9, 13.1, 12.3.

(24 ξ)-Ethyl-6 β -Methoxy-3 α ,5 α -cyclo-stigmast-24-en-26-acetate. A solution of (24 ξ)-Ethyl-6 β -Methoxy-3 α ,5-cyclo-5 α -stigmast-24-en-26-ol (21.3 mg, 0.05 mmol) in 1 mL of 2:1 pyridine/ Ac_2O and

heated overnight at 40 °C. The reaction was evaporated after 15 h under a flow of N₂ and the crude product was filtered through silica gel to obtain 21.5 mg of the desired mixture (92% yield). ¹H NMR (600 MHz): 4.566 (1 H, s), 4.557 (1 H, s), 3.323 (3H, s), 3.320 (3H, s), 2.768 (1H, t, *J* = 2.7 Hz), 2.764 (1H, t, *J* = 2.7 Hz), 2.060 (3H, s), 2.058 (3H, s), 1.682 (3H, s), 1.582 (3H, s), 1.023 (3H, s), 1.019 (3H, s), 0.975 (3H, t, *J* = 7.4 Hz), 0.973 (3H, d, *J* = 6.5 Hz), 0.968 (3H, t, *J* = 7.4 Hz), 1.024 (3H, s), 0.955 (3H, d, *J* = 6.5 Hz), 0.723 (3H, s), 0.712 (3H, s), 0.645 (2H, m), 0.428 (2H, m).

6β-Methoxy-3α,5α-cyclo-stigmast-24-ene (10). To a solution of (24ξ)-Ethyl-6β-Methoxy-3α,5α-cyclo-5α-stigmast-24-en-26-acetate (21.5 mg, 0.040 mmol) in 1 mL of THF was added 0.5 cm of Li wire in 5 mL of condensed NH₃. After 30 min, 150 μL of tBuOH was added to reaction and the mixture was stirred for an additional 30 min before warming it to room temperature. The reaction was extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc and the organic layer was washed with brine and dried over Na₂SO₄ before evaporation under reduced pressure. The crude product was purified with silica gel chromatography to afford 19.2 mg of desired product (96% yield). ¹H NMR (600 MHz): 3.325 (3H, s), 2.768 (1H, t, *J* = 2.7 Hz), 1.632 (3H, s), 1.023 (3H, s), 0.963 (3H, d, *J* = 6.6 Hz), 0.932 (3H, t, *J* = 7.4 Hz), 0.720 (3H, s), 0.646 (1H, dd, *J* = 4.0, 4.9 Hz), 0.429 (1H, dd, *J* = 5.0, 7.9 Hz).

24(ξ),25-Dichloromethano-6β-methoxy-3α,5α-cyclo-stigmastane (11). To solution of **10** (17.1 mg, 0.040 mmol) and benzyltriethylammonium chloride (BTEAC) (8.1 mg, 0.04 mmol) in 1.5 mL of CHCl₃ was added 1mL of 50% aqueous KOH. The mixture was stirred vigorously under a N₂ atmosphere at rt for 16 h. The reaction was extracted with water and 4:1 hexanes/EtOAc. The organic layer was dried over Na₂SO₄, filtered through silica gel and evaporated under reduced pressure. Purification via silica gel chromatography with 39:1 hexanes/EtOAc gave 20.1 mg the desired products (99% yield) ¹H NMR (600 MHz): 3.324 (6H, s), 2.770 (2H, t, *J* = 2.6 Hz), 1.236 (3H, s), 1.232 (3H, s), 1.224 (6H, s), 1.021 (6H, s), 0.950 (3H, d, *J* = 6.6 Hz), 0.6947 (3H, t, *J* = 7.4 Hz), 0.942 (3H, d, *J* = 6.6 Hz), 0.927 (3H, t, *J* = 7.4 Hz), 0.722 (3H, s), 0.717 (3H, s), 0.676 (2H, m), 0.432 (2H, dd, *J* = 5.1, 8.0 Hz).

24(ξ),25-Dichloromethano-stigmast-5-en-3β-ol (12). Compound **11** (20.1 mg, 0.04 mmol) was treated with 1 mL of 1% solution of TFA in DCM for 8 min. 500 μL of 20% methanolic TEA was then added dropwise to reaction before heating the mixture for 1 h at 45 °C. The solvent was evaporated under a N₂ atmosphere and filtered through silica gel with 2:1 hexanes/EtOAc to obtain 19.0 mg of a crude mixture of **12a** and **12b** (98% yield). A sample of the mixture was separated via HPLC to obtain pure samples of **12a** and **12b**. **(24S)-Dichloromethano-stigmast-5-en-3β-ol (12a).** ¹H NMR (800 MHz): 5.352 (1H, m), 3.523 (1H, m), 1.232 (3H, s), 1.224 (3H, s), 1.008 (3H, s), 0.948 (3H, d, *J* = 6.6 Hz), 0.925 (3H, t, *J* = 7.4 Hz), 0.685 (3H, s). **(24R)-Dichloromethano-stigmast-5-en-3β-ol (12b).** ¹H NMR (800 MHz): 5.351 (1H, m), 3.524 (1H, m), 1.235 (3H, s), 1.223 (3H, s), 1.008 (3H, s), 0.956 (3H, d, *J* = 6.6 Hz), 0.946 (3H, t, *J* = 7.4 Hz), 0.680 (3H, s).

24(ξ),25-Dichloromethano-6β-methoxy-3α,5α-cyclo-ergostane (19). The reaction was carried out as described for **11**. Compound **18** (71.3 mg, 0.17 mmol) was used to obtain 81.6 mg of the desired products (95%). ¹H NMR (600 MHz): 3.323 (6H, br. s), 2.769 (2H, m), 1.235 (6H, s), 1.235 (6H, s), 1.195 (6H, s), 1.171 (6H, s), 1.021 (6H, s), 0.931 (3H, d, *J* = 6.5 Hz), 0.924 (3H, d, *J* = 6.5 Hz), 0.719 (3H, s), 0.713 (3H, s), 0.646 (2H, m), 0.431 (2H, dd, *J* = 5.1, 7.9 Hz).

24(ξ),25-Dichloromethano-ergost-5-en-3β-ol (20). The reaction was carried out as described for compound **12**. Compound **19** (80.6 mg, 0.17 mmol) was treated with 3 mL of 1% TFA in DCM and 1 mL of 20% methanolic TEA was used to saponify the TFA esters. A silica gel filtration of the crude product with 4:1 hexanes/EtOAc provided 66.4 mg of a mixture of **20a** and **20b** (85% yield). **(24S)-Dichloromethano-ergost-5-en-3β-ol (20a).** ¹H NMR (800 MHz): 5.352 (1H, m), 3.522 (1H, m), 1.235 (3H, s), 1.196 (3H, s), 1.171 (3H, s), 1.009 (3H, s), 0.931 (3H, d, *J* = 6.6 Hz), 0.685 (3H, s). **(24R)-Dichloromethano-ergost-5-en-3β-ol (20b).** ¹H NMR (800 MHz): 5.352 (1H, m), 3.524 (1H, m), 1.236 (3H, s), 1.197 (3H, s), 1.172 (3H, s), 1.009 (3H, s), 0.940 (3H, d, *J* = 6.5 Hz), 0.680 (3H, s).

General method for the reduction of the dichlorocyclopropanes. To a mixture of 0.25 cm of Li wire in 3 mL of NH₃ was added the dichlorocyclopropane i-methyl ether (under 5 mg) in 1 mL of Et₂O. The reaction was stirred for 1 h before removing the dry ice condenser to allow the NH₃ to evaporate. After extraction with 10% hydrochloric acid and 4:1 hexanes/EtOAc, the organic layer was washed with brine and dried over Na₂SO₄ before evaporation under a N₂ atmosphere. The crude product was purified via TLC with 4:1 hexanes/EtOAc. The obtained product was characterized by NMR.

23-[(1R)-Ethyl-2,2-dimethylcyclopropyl]- 24-norchol-5-en-3 β -ol (13) from 12a. ¹H NMR (800 MHz): 5.351(1H, m), 3.523 (1H, m), 1.093 (3H, s), 1.087 (3H, s), 1.007 (3H, s), 0.914 (3H, d, *J* = 6.6 Hz), 0.871(3H, t, *J* = 7.5 Hz), 0.678 (3H, s), 0.028 (2H, s).

23-[(1S)-Ethyl-2,2-dimethylcyclopropyl]- 24-norchol-5-en-3 β -ol (15) from 12b. ¹H NMR (800 MHz): 5.351(1H, m), 3.523 (1H, m), 1.099 (3H, s), 1.085 (3H, s), 1.007 (3H, s), 0.913 (3H, d, *J* = 6.6 Hz), 0.881(3H, t, *J* = 7.4 Hz), 0.673 (3H, s), 0.015 (2H, dd, *J* = 4.0, 15.4 Hz).

23-[(1R)-1,2,2-trimethylcyclopropyl]- 24-norchol-5-en-3 β -ol (21) from 20a. ¹H NMR (600 MHz): 5.352 (1H, m), 3.524 (1H, m), 1.084 (3H, s), 1.062 (3H, s), 1.171 (3H, s), 1.023 (3H, s), 1.010 (3H, s), 0.905 (3H, d, *J* = 6.6 Hz), 0.680 (3H, s), 0.088 (1H, d, *J* = 3.9 Hz), 0.043 (1H, d, *J* = 3.9 Hz).

23-[(1S)-1,2,2-trimethylcyclopropyl]- 24-norchol-5-en-3 β -ol (22) from 20b. ¹H NMR (600 MHz): 5.353 (1H, m), 3.524 (1H, m), 1.087 (3H, s), 1.065 (3H, s), 1.171 (3H, s), 1.023 (3H, s), 1.010 (3H, s), 0.906 (3H, d, *J* = 6.6 Hz), 0.675 (3H, s), 0.074 (1H, d, *J* = 3.9 Hz), 0.043 (1H, d, *J* = 3.9 Hz).

General method for the TFA-catalyzed ring opening of sidechain cyclopropanes. Approximately 0.5 to 1 mg of each substrate was treated with 500 μ L 2% TFA in dry DCM overnight. A solution of 20% methanolic TEA (500 μ L) was then added dropwise to reaction before heating the mixture for 1 h at 45

°C. The solvent was evaporated under a N₂ atmosphere and purified by preparative TLC with 4:1 hexanes/EtOAc. HPLC purification was necessary to separate the desired product from any remaining starting material and minor side products.

(24R)-24Ethyl-24-methyl-cholesta-5,25-dien-3-ol (14) from 13. ¹H NMR (800 MHz): 5.349 (1 H, m), 4.812 (1 H, br s), 4.620 (1H, d, *J* = 1.5 Hz), 3.520 (1H, m), 1.621 (3H, br s), 1.007 (3H, s), 0.930 (3H, s), 0.912 (3H, d, *J* = 6.6 Hz), 0.711 (3H, t, *J* = 7.4 Hz), 0.668 (1H, s).

(24S)-24Ethyl-24-methyl-cholesta-5,25-dien-3-ol (16) from 15. ¹H NMR (800 MHz): 5.349 (1 H, m), 4.801(1 H, br s), 4.623 (1H, d, *J* = 1.6 Hz), 3.520 (1H, m), 1.629 (3H, br s), 1.006 (3H, s), 0.941 (3H, s), 0.912 (3H, d, *J* = 6.6 Hz), 0.716 (3H, t, *J* = 7.4 Hz), 0.671 (1H, s).

(24S)-24-Deutero-methyl-ergost-5,25-dien-3-ol (23) (minor) from 21. A solution of 2% deuterated TFA was used instead of non-deuterated TFA in the ring opening reaction. ¹H NMR (800 MHz): 5.350 (1 H, m), 4.718 (1 H, br s), 4.656 (1H, s), 3.522 (1H, m), 1.684 (3H, br s), 1.013 (3H, s), 1.009 (3H, s), 0.912 (3H, d, *J* = 6.6 Hz), 0.672 (1H, s). ¹³C NMR (201 MHz): 152.4, 140.8, 121.7, 109.3, 71.8, 56.8, 55.9, 50.2, 42.4, 43.3, 39.1 (2C), 38.6 (C24, Δ_D = 70 ppb), 37.3, 37.0 (C23, Δ_D = 20 ppb), 36.5, 36.2, 31.9, 31.7, 30.5, 28.2, 27.5 (C28, 24α-Me), 26.9 (24β-Me, CHD₂, Δ_D = 320 ppb, t, *J* = 19.2Hz), 24.3, 21.1, 19.40 (C27), 19.39 (C19), 18.9 (C21), 11.8 (C18).

(24R)-24-Deutero-methyl-ergost-5,25-dien-3-ol (24) (minor) from 22. A solution of 2% deuterated TFA was used instead of non-deuterated TFA in the ring opening reaction. ¹H NMR (800 MHz): 5.350 (1 H, m), 4.718 (1 H, br s), 4.656 (1H, s), 3.522 (1H, m), 1.684 (3H, br s), 1.013 (3H, s), 1.009 (3H, s), 0.912 (3H, d, *J* = 6.6 Hz), 0.672 (1H, s). ¹³C NMR (201 MHz): 152.4, 140.8, 121.7, 109.3, 71.8, 56.8, 55.9, 50.2, 42.4, 43.3, 39.1 (2C), 38.7 (C24, Δ_D = 70 ppb), 37.3, 37.0 (C23, Δ_D = 20 ppb), 36.5, 36.2, 31.9,

31.7, 30.5 (C22) , 28.2, 27.2 (24 α -Me, CHD₂, Δ_D = 318 ppb, t, J = 19.5 Hz), 27.2 (C28, 24 β -Me, Δ_D = 21 ppb), 24.3, 21.1, 19.4 (C19 and C27), 18.9 (C21), 11.8 (C18).

General method for the hydrogenation of unsaturated i-methyl ethers. To a stirred suspension of about 5 mg of 10% w/w Pd/C in 500 μ L EtOAc under H₂ was added the dissolved starting material (approximately 1 mg) was in 1 mL of EtOAc. The reaction was stirred for 20 min before removing the catalyst via silica gel filtration. The solvent was evaporated, and the crude product was purified via preparative TLC before NMR analysis.

(24R)-24Ethyl-24-methyl-cholestan-3-ol (14) from 3. ¹H NMR (600 MHz): 3.587 (1 H, m), 0.907 (3H, d, J = 6.6 Hz), 0.802 (1H, s), 0.796 (3H, d, J = 6.6 Hz), 0.785 (3H, d, J = 6.6 Hz), 0.748 (3H, t, J = 7.5 Hz), 0.672 (1H, s), 0.648 (1H, s).

(24S)-24Ethyl-24-methyl-cholestan-3-ol (16) from 17. ¹H NMR (600 MHz): 3.587 (1 H, m), 0.908 (3H, d, J = 6.6 Hz), 0.803 (1H, s), 0.794 (3H, d, J = 7.0 Hz), 0.790 (3H, d, J = 6.9 Hz), 0.753 (3H, t, J = 7.5 Hz), 0.671 (1H, s), 0.648 (1H, s).

General method for the dichlorocyclopropanation of the allylic alcohol i-methyl ethers. To solution of the starting material (0.5-5 mg) and an excess of benzyltriethylammonium chloride (BTEAC) (4.2 mg, 0.02 mmol) in 0.5 mL of CHCl₃ was added 0.5 mL of 50% aqueous KOH. The mixture was stirred vigorously under a N₂ atmosphere at room temperature for 75 min. The reaction was extracted with water and 4:1 hexanes/EtOAc. The organic layer was dried over Na₂SO₄, filtered through silica gel and evaporated under a flow of N₂. The crude product was purified with preparative TLC with 9:1 hexanes/EtOAc before HPLC separation with methanol to separate the mixture of diastereomers.

(24S,25S)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-stigmastan-26-ol (25a) from 9a. ^1H NMR (600 MHz): 3.833 (1H, d, $J = 11.8$ Hz), 3.738 (1H, d, $J = 11.8$), 3.326 (3H, s), 2.771 (1H, t, $J = 2.8$ Hz), 1.347 (3H, s), 1.024 (3H, s), 0.945 (3H, d, $J = 6.6$ Hz), 0.942 (3H, t, $J = 7.4$ Hz), 0.722 (3H, s), 0.650 (1H, dd, $J = 4.1, 4.8$ Hz), 0.434 (1H, dd, $J = 5.1, 8.0$).

(24R,25R)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-stigmastan-26-ol (25b) from 9a. ^1H NMR (600 MHz): 3.832 (1H, d, $J = 11.8$ Hz), 3.740 (1H, d, $J = 11.8$ Hz), 3.327 (3H, s), 2.771 (1H, d, $J = 2.8$ Hz), 1.349 (3H, s), 1.023 (3H, s), 0.959 (3H, t, $J = 7.4$ Hz), 0.953 (3H, d, $J = 6.6$ Hz), 0.717 (3H, s), 0.650 (1H, dd, $J = 4.1, 4.8$ Hz), 0.435 (1H, dd, $J = 5.1, 8.0$ Hz).

(24S, 25R)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-stigmastan-26-ol (26a) from 9b. ^1H NMR (600 MHz): 3.824 (1H, d, $J = 11.8$ Hz), 3.738 (1H, d, $J = 11.8$ Hz), 3.325 (3H, s), 2.771 (1H, t, $J = 2.8$ Hz), 1.356 (3H, s), 1.022 (1H, s), 0.949 (3H, t, $J = 7.4$ Hz), 0.946 (3H, d, $J = 6.6$ Hz), 0.723 (3H, s), 0.653 (1H, dd, $J = 4.1, 4.8$), 0.433 (1H, dd, $J = 5.1, 8.0$ Hz).

(24R, 25S)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-stigmastan-26-ol (26b) from 9b. ^1H NMR (600 MHz): 3.827 (1H, d, $J = 11.8$ Hz), 3.741 (1H, d, $J = 11.8$ Hz), 3.325 (3H, s), 2.771 (1H, t, $J = 2.8$ Hz), 1.355 (3H, s), 1.022 (3H, s), 0.974 (1H, t, $J = 7.4$ Hz), 0.955 (3H, d, $J = 6.6$ Hz), 0.719 (1H, s), 0.649 (1H, dd, $J = 4.0, 4.8$ Hz), 0.433 (1H, dd, $J = 5.1, 8.0$ Hz).

(24S, 25S)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-ergostan-26-ol (52a) from 51a. ^1H NMR (600 MHz): 3.840 (1H, d, $J = 11.8$ Hz), 3.739 (1H, d, $J = 11.9$ Hz), 3.325 (3H, s), 2.770 (1H, t, $J = 2.7$ Hz), 1.328 (3H, s), 1.198 (3H, s), 1.023 (3H, s), 0.928 (3H, d, $J = 6.4$ Hz), 0.720 (3H, s), 0.649 (1H, dd, $J = 4.2, 4.8$ Hz), 0.434 (1H, dd, $J = 5.1, 8.0$ Hz).

(24R, 25R)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-ergostan-26-ol (52b) from 51a. ^1H NMR (600 MHz): 3.830 (1H, d, $J = 11.8$ Hz), 3.737 (1H, d, $J = 11.8$ Hz), 3.326 (3H, s), 2.770 (1H, t, $J = 2.7$ Hz), 1.328 (3H, s), 1.196 (3H, s), 1.023 (3H, s), 0.935 (3H, d, $J = 6.4$ Hz), 0.714 (3H, s), 0.649 (1H, dd, $J = 4.1, 4.7$ Hz), 0.434 (1H, dd, $J = 5.1, 8.0$ Hz).

(24S,25R)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-ergostan-26-ol (53a) from 51b. ^1H NMR (600 MHz): 3.790 (1H, d, $J = 11.7$ Hz), 3.694 (1H, d, $J = 11.7$ Hz), 3.325 (3H, s), 2.77 (1H, t, $J = 2.7$ Hz), 1.351 (3H, s), 1.271 (3H, s), 1.023 (3H, s), 0.931 (3H, d, $J = 6.4$ Hz), 0.721 (3H, s), 0.649 (1H, dd, $J = 4.1, 4.7$ Hz), 0.434 (1H, dd, $J = 5.1, 8.0$ Hz).

(24R,25S)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-ergostan-26-ol (53b) from 51b. ^1H NMR (600 MHz): 3.784 (1H, d, $J = 11.7$ Hz), 3.689 (1H, d, $J = 11.7$ Hz), 3.326 (3H, s), 2.771 (1H, t, $J = 2.8$ Hz), 1.350 (3H, s), 1.270 (1H, s), 1.024 (1H, s), 0.939 (3H, d, $J = 6.5$ Hz), 0.718 (3H, s), 0.650 (1H, dd, $J = 4.2, 4.8$ Hz), 0.435 (1H, dd, $J = 5.1, 8.0$ Hz).

(24S,25S)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergostan-26-ol (75a) from 74a. ^1H NMR (600 MHz): 3.860 (1H, dd, $J = 5.8, 12.1$ Hz), 3.819 (1H, m), 3.325 (3H, s), 2.770 (1H, t, $J = 2.7$ Hz), 1.222 (3H, s), 1.023 (3H, s), 0.995 (3H, t, $J = 7.4$ Hz), 0.927 (3H, d, $J = 6.6$ Hz), 0.719 (3H, s), 0.649 (1H, dd, $J = 4.2, 4.8$ Hz), 0.434 (1H, dd, $J = 5.1, 8.0$ Hz).

(24R,25R)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergostan-26-ol (75b) from 74a. ^1H NMR (600 MHz): 3.869 (1H, m), 3.796 (1H, m), 3.326 (3H, s), 2.771 (1H, t, $J = 2.8$ Hz), 1.218 (3H, s), 0.992 (3H, t, $J = 7.4$ Hz), 0.933 (3H, d, $J = 6.6$ Hz), 0.714 (3H, s), 0.650 (1H, dd, $J = 4.1, 4.8$ Hz), 0.435 (1H, dd, $J = 5.1, 8.0$ Hz).

(24*S*,25*R*)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergostan-26-ol (76a) from 74b. ¹H NMR (600 MHz): 3.886 (1H, m), 3.762 (1H, m), 3.326 (3H, s), 2.771 (1H, t, *J* = 2.7 Hz), 1.272 (3H, s), 1.059 (3H, t, *J* = 7.4 Hz), 1.024 (3H, s), 0.930 (3H, d, *J* = 6.6 Hz), 0.722 (3H, s), 0.649 (1H, dd, *J* = 4.0, 4.8 Hz), 0.434 (1H, dd, *J* = 5.1, 8.0 Hz).

(24*R*,25*S*)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergostan-26-ol (76b) from 74b. ¹H NMR (600 MHz): 3.889 (3H, dd, *J* = 5.1, 12.1 Hz), 3.749 (3H, dd, *J* = 7.2, 12.1 Hz), 3.326 (3H, s), 2.771 (1H, t, *J* = 2.7 Hz), 1.280 (3H, s), 1.073 (3H, t, *J* = 7.4 Hz), 1.023 (3H, s), 0.940 (3H, d, *J* = 6.6 Hz), 0.719 (1H, s), 0.649 (3H, dd, *J* = 4.1, 4.8 Hz), 0.434 (1H, dd, *J* = 5.1, 8.0 Hz).

23-[(1*R*,2*R*)-(1-Ethyl-2-methylcyclopropanemethanol)]-6 β -Methoxy-3 α ,5 α -cyclo-24-norcholane (27).

Compound **26a** (6.2 mg, 0.012 mmol) was dechlorinated as described above in the general methods for the reduction of the dichlorocyclopropanes to obtain 5.0 mg **27** (92% yield). ¹H NMR (600 MHz): 3.616 (1H, d, *J* = 11.3 Hz), 3.534 (1H, d, *J* = 11.3 Hz), 3.326 (3H, s), 2.770 (1H, t, *J* = 2.8 Hz), 1.225 (3H, s), 1.025 (3H, s), 0.925 (3H, d, *J* = 6.6 Hz), 0.910 (3H, t, *J* = 7.4 Hz), 0.721 (3H, s), 0.648 (1H, dd, *J* = 4.0, 4.8 Hz), 0.433 (1H, dd, *J* = 5.0, 8.0 Hz), 0.274 (1H, d, *J* = 4.4 Hz), 0.131 (1H, d, *J* = 4.4 Hz).

General method for the DMP oxidation of the substituted cyclopropanemethanol i-methyl ethers.

To a solution of approximately 0.5-2 mg of alcohol i-methyl ether in 1 mL of DCM was added approximately 2.6-4.3 mg of DMP. The reaction was stirred for 1 h and checked by analytical TLC before filtering the crude extract with 9:1 hexanes/EtOAc through 4 cm of silica gel in a Pasteur pipet. The solvent was evaporated under a flow of N₂ and purified via preparative TLC with 19:1 hexanes/EtOAc. At least an 80% yield was observed for each reaction.

23-[(1*R*,2*R*)-(1-Ethyl-2-methylcyclopropanecarboxaldehyde)]-6 β -Methoxy-3 α ,5 α -cyclo-24-

norcholane (28). **28** was obtained from **27** as described above in the general method for the DMP

oxidation. ¹H NMR (600 MHz): 9.298 (1H, s), 3.327 (3H, s), 2.773 (1H, t, *J* = 2.8 Hz), 1.284 (3H, s), 1.025 (3H, s), 0.927 (3H, d, *J* = 6.6 Hz), 0.867 (3H, t, *J* = 7.4 Hz), 0.724 (3H, s), 0.651 (1H, dd, *J* = 4.0, 4.8 Hz), 0.435 (1H, d, *J* = 5.1, 8.0 Hz).

General method for the methylenation of the substituted cyclopropanecarboxaldehyde i-methyl ethers. A mixture of methyltriphenylphosphonium iodide (72.8 mg, 0.200 mmol) and KtBuO (19.2 mg, 0.170 mmol) in 3 mL of dry THF was heated to reflux for under a N₂ atmosphere for 30 min. The resulting suspension was cooled to rt before adding 1 mL of the supernatant (ylide) to a solution of the cyclopropanecarboxaldehyde i-methyl ether (1-5 mg) in 500 μL of THF. The mixture was stirred under a N₂ atmosphere for 20 min before extraction with water and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under a flow of N₂. The crude product was purified by preparative TLC with 3:1 hexanes/DCM.

23-[(1*R*, 2*S*)-(1-Ethyl-2-ethenyl-2-methylcyclopropyl)]-6β-Methoxy-3*α*,5*α*-cyclo-24-norcholane (29).

29 was obtained from **28** as described above in the general method for the methylenation of the substituted cyclopropanecarboxaldehyde i-methyl ethers. ¹H NMR (600 MHz): 5.838 (1H, dd, *J* = 10.1, 17.8 Hz), 4.976 (1H, dd, *J* = 1.7, 5.7 Hz), 4.953 (1H, m), 3.325 (3H, s), 3.770 (1H, t, *J* = 2.8 Hz), 1.215 (3H, s), 1.024 (3H, s), 0.922 (3H, d, *J* = 6.6 Hz), 0.836 (3H, t, *J* = 7.4 Hz), 0.720 (3H, s), 0.649 (1H, dd, *J* = 4.0, 4.8 Hz), 0.569 (1H, d, *J* = 4.4 Hz), 0.431 (1H, dd, *J* = 5.1, 8.0 Hz), 0.319 (1H, d, *J* = 4.4 Hz).

23-[(1*R*, 2*S*)-(1-Ethyl-2-methylcyclopropaneethanol)]-6β-Methoxy-3*α*,5*α*-cyclo-24-norcholane (30).

To a solution of **29** (1 mg, 0.002 mmol) in 900 μL of THF was added 100 μL 2 M BMS dropwise under a N₂ atmosphere. The reaction was stirred for 2 h before adding 10 drops of 10% methanolic NaOH dropwise, followed by 10 drops of 30% H₂O₂. The reaction was stirred at room temperature for 3 h before extracting the product with water and 4:1 hexanes/EtOAc. The organic layer was washed with brine, dried over Na₂SO₄ and evaporated under a flow of N₂. The crude product was purified by preparative TLC with

4:1 hexanes/EtOAc. ¹H NMR (600 MHz): 3.739 (2H, m), 3.326 (3H, s), 2.769 (1H, t, *J* = 2.8 Hz), 1.111 (3H, s), 1.023 (3H, s), 0.913 (3H, d, *J* = 6.6 Hz), 0.891 (3H, t, *J* = 7.4 Hz), 0.716 (3H, s), 0.648 (1H, dd, *J* = 4.0, 4.8 Hz), 0.432 (1H, dd, *J* = 5.1, 8.0 Hz), 0.160 (1H, d, *J* = 4.2 Hz), 0.066 (1H, t, *J* = 4.2 Hz).

23-[(1*R*,2*S*)-(1-Ethyl-2-[(methylsulfonyl)oxy]ethyl)-2-methylcyclopropyl]-6β-Methoxy-3α,5α-cyclo-24-norcholane (31). To a solution of **30** (approximately 1 mg, 0.002 mmol) and 750 μL of dry 1% TEA in DCM was added 250 μL of 1% MsCl in dry DCM at 0 °C. The reaction was stirred for 30 min at 0 °C before extraction with water and 4:1 hexanes/EtOAc. The organic layer was washed with 5% hydrochloric acid followed by a brine wash and dried over Na₂SO₄. The crude was evaporated under a flow of N₂ and purified with preparative TLC with 4:1 hexanes/EtOAc. ¹H NMR (600 MHz): 4.291 (2H, m), 3.326 (1H, s), 3.002 (3H, s), 2.770 (1H, t, *J* = 2.8 Hz), 1.128 (3H, s), 1.024 (3H, s), 0.912 (3H, d, *J* = 6.6 Hz), 0.897 (3H, t, *J* = 7.4 Hz), 0.716 (3H, s), 0.648 (1H, dd, *J* = 4.0, 4.8 Hz), 0.432 (1H, dd, *J* = 5.1, 8.0 Hz), 0.193 (1H, d, *J* = 4.5 Hz), 0.112 (1H, d, *J* = 4.4 Hz).

23-[(1*R*,2*R*)-(1,2-Diethyl-2-methylcyclopropyl)-6β-Methoxy-3α,5α-cyclo-24-norcholane (32). A solution of **31** (approximately 1 mg, 0.002 mmol) in 1.5 mL of Et₂O was treated with 3.6 mg of LAH. The mixture was stirred for 30 min at room temperature and the reaction was extracted with 10% hydrochloric acid and 4:1 hexanes/EtOAc. The organic layer was washed with brine and dried over Na₂SO₄. The crude product was purified with preparative TLC with 39:1 hexanes/EtOAc. ¹H NMR (600 MHz): 3.326 (3H, s), 2.769 (1H, t, *J* = 2.8 Hz), 1.065 (3H, s), 1.024 (3H, s), 0.915 (3H, d, *J* = 6.6 Hz), 0.897 (3H, d, *J* = 7.4 Hz), 0.876 (3H, t, *J* = 7.4 Hz), 0.718 (3H, s), 0.648 (1H, dd, *J* = 4.0, 4.9 Hz), 0.431 (1H, dd, *J* = 5.1, 8.0 Hz), 0.045 (1H, d, *J* = 4.0 Hz), -0.010 (1H, d, *J* = 4.0 Hz).

General method for the deprotection of i-methyl ethers. Approximately 1-5 mg of i-methyl ether was treated with 500 μL of 1% TFA in DCM for 8 min. A solution of 100 μL of 20% methanolic TEA was then added dropwise to the reaction before heating the mixture for 1 h at 45 °C. The solvent was

evaporated under a N₂ atmosphere and the crude product was purified by preparative TLC with 4:1 hexanes/EtOAc.

23-[(1*R*,2*R*)-(1,2-Diethyl-2-methylcyclopropyl)-24-norchol-5-en-3 β -ol (33). Compound **33** was obtained from **32** as described above in the general method for the deprotection of i-methyl ethers. The product was further purified by HPLC before the characterization by NMR. ¹H NMR (800 MHz): 5.353 (1H, m), 3.523 (1H, m), 1.065 (3H, s), 1.012 (3H, s), 0.932 (3H, d, *J* = 6.6 Hz), 0.897 (3H, t, *J* = 7.4 Hz), 0.876 (3H, t, *J* = 7.4 Hz), 0.684 (3H, s), 0.047 (1H, d, *J* = 4.0 Hz), -0.009 (1H, t, *J* = 4.0 Hz).

(24*S*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-stigmastan-26-al (34). Compound **34** was obtained from **25a** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ¹H NMR (600 MHz): 9.632 (1H, s), 3.326 (3H, s), 2.771 (1H, t, *J* = 2.8 Hz), 1.315 (3H, s), 1.022 (3H, s), 0.982 (3H, t, *J* = 7.4 Hz), 0.936 (3H, d, *J* = 6.5 Hz), 0.719 (3H, s), 0.649 (1H, dd, *J* = 4.1, 4.9 Hz), 0.434 (1H, dd, *J* = 5.1, 8.0 Hz).

(24*R*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-stigmastan-26-al (35). Compound **35** was obtained from **25b** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ¹H NMR (600 MHz): 9.616 (1H, s), 3.323 (3H, s), 2.768 (1H, t, *J* = 2.8 Hz), 1.315 (3H, s), 1.020 (3H, s), 1.004 (3H, t, *J* = 7.4 Hz), 0.945 (3H, d, *J* = 6.5 Hz), 0.706 (3H, s), 0.648 (1H, dd, *J* = 4.1, 4.8 Hz), 0.433 (1H, dd, *J* = 5.1, 8.0 Hz).

(24*R*,25*S*)-Dichloromethano-6 β -methoxy-3 α , α 5-cyclo-stigmastan-26-al (36). Compound **36** was obtained from **26b** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ¹H NMR (600 MHz): 9.628 (1H, s), 3.327 (3H, s), 2.772 (1H, t, *J* = 2.8 Hz), 1.328 (3H, s), 1.023 (3H, s), 0.994 (3H, t, *J* = 7.4 Hz), 0.956 (3H, d, *J* = 6.5 Hz), 0.720 (3H, s), 0.650 (1H, dd, *J* = 4.1, 4.8 Hz), 0.436 (1H, t, *J* = 5.1, 8.0 Hz).

(24*S*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-ergostan-26-ol (54). Compound **54** was obtained from **52a** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ¹H NMR (600 MHz): 9.610 (1H, s), 3.324 (3H, s), 2.769 (1H, t, *J* = 2.7 Hz), 1.292 (3H, s), 1.280 (3H, s), 1.020 (3H, s), 0.920 (3H, d, *J* = 6.5 Hz), 0.716 (3H, s), 0.648 (1H, dd, *J* = 4.1, 4.6 Hz), 0.433 (1H, dd, *J* = 5.2, 8.1).

(24*R*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-ergostan-26-ol (55). Compound **55** was obtained from **52b** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ¹H NMR (600 MHz): 9.588 (1H, s), 3.324 (3H, s), 2.767 (1H, t, *J* = 2.7 Hz), 1.292 (3H, s), 1.276 (3H, s), 1.020 (3H, s), 0.931 (3H, d, *J* = 6.5 Hz), 0.704 (3H, s), 0.648 (1H, t, *J* = 4.1, 4.7 Hz), 0.434 (1H, t, *J* = 5.1, 8.0 Hz).

(24*S*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-ergostan-26-al (56). Compound **56** was obtained from **53a** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ¹H NMR (600 MHz): 9.597 (1H, s), 3.327 (3H, s), 2.772 (1H, t, *J* = 2.8 Hz), 1.530 (3H, s), 1.334 (3H, s), 1.024 (3H, s), 0.931 (3H, d, *J* = 6.6 Hz), 0.723 (3H, s), 0.651 (1H, t, *J* = 4.1, 4.7 Hz), 0.436 (1H, t, *J* = 5.1, 8.0 Hz).

(24*R*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-ergostan-26-al (57). Compound **57** was obtained from **53b** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ¹H NMR (600 MHz): 9.583 (1H, s), 3.326 (3H, s), 2.772 (1H, t, *J* = 2.7 Hz), 1.534 (3H, s), 1.333 (3H, s), 1.022 (3H, s), 0.939 (3H, d, *J* = 6.6 Hz), 0.715 (3H, s), 0.650 (1H, dd, *J* = 4.1, 4.7 Hz), 0.436 (1H, dd, *J* = 5.1, 8.0 Hz).

(24*S*,25*S*)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergostan-26-al (77). Compound **77** was obtained from **75a** as described above in the general method for DMP oxidation of the cyclopropane

alcohols. ^1H NMR (600 MHz): 9.581 (1H, s), 3.324 (3H, s), 2.769 (1H, t, $J = 2.7$ Hz), 1.315 (3H, s), 1.020 (3H, s), 0.972 (3H, t, $J = 7.3$ Hz), 0.916 (3H, d, $J = 6.6$ Hz), 0.714 (3H, s), 0.648 (1H, dd, $J = 4.1$, 5.0 Hz), 0.433 (1H, dd, $J = 5.1$, 8.0 Hz).

(24*R*,25*R*)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergostan-26-al (78). Compound **78** was obtained from **75b** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ^1H NMR (600 MHz): 9.553 (1H, s), 3.324 (3H, s), 2.769 (1H, t, $J = 2.8$ Hz), 1.312 (3H, s), 1.020 (3H, s), 0.970 (1H, t, $J = 7.4$ Hz), 0.927 (3H, d, $J = 6.6$ Hz), 0.704 (3H, s), 0.648 (1H, dd, $J = 4.1$, 5.1 Hz), 0.434 (1H, dd, $J = 5.1$, 7.8 Hz).

(24*S*,25*R*)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergostan-26-al (79). Compound **79** was obtained from **76a** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ^1H NMR (600 MHz): 9.609 (1H, d, $J = 1.0$ Hz), 3.327 (3H, s), 2.773 (1H, t, $J = 2.8$ Hz), 1.437 (3H, s), 1.023 (3H, s), 1.006 (3H, t, $J = 7.3$ Hz), 0.928 (3H, d, $J = 6.5$ Hz), 0.721 (3H, s), 0.650 (1H, dd, $J = 4.1$, 4.9 Hz), 0.436 (1H, dd, $J = 5.1$, 8.0 Hz).

(24*R*,25*S*)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-27-homoergostan-26-al (80). Compound **80** was obtained from **76b** as described above in the general method for DMP oxidation of the cyclopropane alcohols. ^1H NMR (600 MHz): 9.601 (1H, d, $J = 0.9$ Hz), 3.327 (3H, s), 2.773 (1H, t, $J = 2.8$ Hz), 1.434 (3H, s), 1.023 (3H, s), 1.016 (3H, t, $J = 7.4$ Hz), 0.936 (3H, d, $J = 6.6$ Hz), 0.717 (3H, s), 0.650 (1H, dd, $J = 4.1$, 4.8 Hz), 0.436 (1H, dd, $J = 5.1$, 8.1 Hz).

(24*S*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homostigmast-26-ene (37). Compound **37** was obtained from **34** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.999 (1H, dd, $J = 10.9$, 17.4 Hz), 5.277 (1H, dd, $J = 0.9$, 10.9 Hz), 5.247 (1H, t, $J = 0.9$, 17.4 Hz), 3.325 (3H, s), 2.770 (1H, t, $J = 2.7$ Hz), 1.359 (3H, s),

1.022 (3H, s), 0.944 (3H, t, $J = 7.4$ Hz), 0.926 (3H, d, $J = 6.6$ Hz), 0.715 (3H, s), 0.648 (1H, dd, $J = 3.9, 4.7$ Hz), 0.433 (1H, dd, $J = 5.1, 7.9$ Hz).

(24*R*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homostigmast-26-ene (38). Compound **38** was obtained from **35** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.995 (1H, dd, $J = 10.9, 17.3$ Hz), 5.287 (1H, dd, $J = 1.1, 10.9$ Hz), 5.257 (1H, t, $J = 1.0, 17.4$ Hz), 3.325 (3H, s), 2.769 (1H, t, $J = 2.7$ Hz), 1.358 (3H, s), 1.022 (3H, s), 0.964 (3H, t, $J = 7.4$ Hz), 0.931 (3H, d, $J = 6.6$ Hz), 0.708 (3H, s), 0.649 (1H, dd, $J = 4.0, 4.8$ Hz), 0.432 (1H, dd, $J = 5.0, 8.0$ Hz).

(24*R*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homostigmast-26-ene (39). Compound **39** was obtained from **36** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.986 (1H, dd, $J = 10.9, 17.4$ Hz), 5.269 (1H, dd, $J = 1.1, 10.9$ Hz), 5.328 (1H, dd, $J = 1.1, 17.4$ Hz), 3.327 (3H, s), 2.772 (1H, t, $J = 2.8$ Hz), 1.367 (3H, s), 1.025 (3H, s), 0.961 (3H, d, $J = 6.6$ Hz), 0.933 (3H, t, $J = 7.4$ Hz), 0.722 (3H, s), 0.651 (1H, dd, $J = 4.1, 4.9$ Hz), 0.435 (1H, t, $J = 5.1, 8.0$ Hz).

(24*S*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homoergost-26-ene (58). Compound **58** was obtained from **54** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.991 (1H, dd, $J = 11.0, 17.3$ Hz), 5.276 (1H, dd, $J = 10.9, 11.8$ Hz), 5.259 (1H, dd, $J = 17.3, 18.1$ Hz), 3.324 (3H, s), 2.768 (1H, t, $J = 2.7$ Hz), 1.328 (3H, s), 1.234 (3H, s), 1.022 (3H, s), 0.910 (3H, d, $J = 6.6$ Hz), 0.713 (3H, s), 0.647 (1H, dd, $J = 4.1, 4.8$ Hz), 0.432 (1H, dd, $J = 5.1, 8.0$ Hz).

(24*R*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homoergost-26-ene (59). Compound **59** was obtained from **55** as described above in the general method for the methylenation of the substituted

carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.976 (1H, dd, $J = 10.9, 17.3$ Hz), 5.286 (1H, dd, $J = 10.9, 11.7$ Hz), 5.267 (1H, dd, $J = 17.3, 18.1$ Hz), 3.324 (3H, s), 2.768 (1H, t, $J = 2.7$ Hz), 1.326 (3H, s), 1.234 (3H, s), 1.022 (3H, s), 0.915 (3H, d, $J = 6.6$ Hz), 0.705 (3H, s), 0.648 (1H, dd, $J = 4.1, 4.7$ Hz), 0.432 (1H, dd, $J = 5.1, 7.9$ Hz).

(24*S*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homoergost-26-ene (60). Compound **60** was obtained from **56** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.934 (1H, dd, $J = 10.9, 17.4$ Hz), 5.268 (1H, dd, $J = 10.9, 11.9$ Hz), 5.225 (1H, dd, $J = 17.4, 18.4$ Hz), 3.326 (3H, s), 2.771 (1H, t, $J = 2.7$ Hz), 1.370 (3H, s), 1.215 (3H, s), 1.025 (3H, s), 0.936 (1H, d, $J = 6.6$ Hz), 0.725 (3H, s), 0.649 (1H, dd, $J = 4.2, 4.8$ Hz), 0.435 (1H, dd, $J = 5.1, 7.9$ Hz).

(24*R*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homoergost-26-ene (61). Compound **61** was obtained from **57** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.925 (1H, dd, $J = 10.9, 17.4$ Hz), 5.268 (1H, dd, $J = 10.9, 11.8$ Hz), 5.227 (1H, dd, $J = 17.4, 18.2$ Hz), 3.327 (3H, s), 2.772 (1H, dd, $J = 2.7$ Hz), 1.370 (3H, s), 1.216 (3H, s), 1.024 (3H, s), 0.942 (3H, d, $J = 6.6$ Hz), 0.719 (3H, s), 0.650 (1H, dd, $J = 4.2, 4.8$ Hz), 0.435 (1H, dd, $J = 5.1, 7.8$ Hz).

(24*S*,25*R*)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-26,27-dihomoergost-26-ene (81). Compound **81** was obtained from **77** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.795 (1H, dd, $J = 10.9, 17.7$ Hz), 5.384 (1H, dd, $J = 1.1, 10.9$ Hz), 5.214 (1H, dd, $J = 1.1, 17.7$ Hz), 3.323 (3H, s), 2.769 (1H, t, $J = 2.7$ Hz), 1.247 (3H, s), 1.020 (3H, s), 0.976 (3H, t, $J = 7.3$ Hz), 0.912 (3H, d, $J = 6.6$ Hz), 0.709 (3H, s), 0.646 (1H, dd, $J = 4.1, 5.0$ Hz), 0.430 (1H, t, $J = 5.1, 8.0$ Hz).

(24R,25S)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-26,27-dihomoergost-26-ene (82). Compound **82** was obtained from **78** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.780 (1H, dd, $J = 11.0, 17.6$ Hz), 5.397 (1H, dd, $J = 1.1, 11.0$ Hz), 5.231 (1H, dd, $J = 1.1, 17.7$ Hz), 3.324 (3H, s), 2.769 (1H, t, $J = 2.7$ Hz), 1.247 (3H, s), 1.021 (3H, s), 0.975 (3H, t, $J = 7.4$ Hz), 0.908 (3H, d, $J = 6.6$ Hz), 0.706 (3H, s), 0.648 (1H, dd, $J = 4.1, 4.8$ Hz), 0.431 (1H, dd, $J = 5.2, 8.0$ Hz).

(24S,25S)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-26,27-dihomoergost-26-ene (83). Compound **83** was obtained from **79** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.801 (1H, dd, $J = 10.9, 17.7$ Hz), 5.372 (1H, dd, $J = 1.3, 10.9$ Hz), 5.148 (1H, dd, $J = 1.3, 17.7$ Hz), 3.326 (3H, s), 2.771 (1H, t, $J = 2.8$ Hz), 1.139 (3H, s), 1.026 (3H, s), 1.012 (3H, t, $J = 7.3$ Hz), 0.939 (3H, d, $J = 6.6$ Hz), 0.727 (3H, s), 0.650 (1H, dd, $J = 4.1, 4.8$ Hz), 0.435 (1H, dd, $J = 5.1, 8.0$ Hz).

(24R,25R)-Dichloromethano-6 β -Methoxy-3 α ,5 α -cyclo-26,27-dihomoergost-26-ene (84). Compound **84** was obtained from **80** as described above in the general method for the methylenation of the substituted carboxaldehyde i-methyl ethers. ^1H NMR (600 MHz): 5.808 (1H, dd, $J = 10.9, 17.7$ Hz), 5.371 (1H, dd, $J = 1.3, 10.9$ Hz), 5.145 (1H, dd, $J = 1.3, 17.7$ Hz), 3.328 (3H, s), 2.773 (1H, t, $J = 2.8$ Hz), 1.132 (3H, s), 1.025 (3H, s), 1.022 (3H, t, $J = 7.4$ Hz), 0.945 (3H, d, $J = 6.6$ Hz), 0.723 (3H, s), 0.650 (1H, dd, $J = 4.1, 4.8$ Hz), 0.435 (1H, dd, $J = 5.2, 8.2$ Hz).

(24S,25R)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homostigmastane (40). Compound **40** was obtained from **37** as described above in the general method for the hydrogenation of the unsaturated i-methyl ethers. ^1H NMR (600 MHz): 3.326 (3H, s), 2.771 (1H, t, $J = 2.7$ Hz), 1.201 (3H, s), 1.024 (3H, s), 0.997 (3H, t, $J = 7.4$ Hz), 0.941 (3H, d, $J = 6.6$ Hz), 0.907 (3H, t, $J = 7.4$ Hz), 0.723 (3H, s), 0.650 (1H, dd, $J = 4.1, 4.8$ Hz), 0.433 (1H, dd, $J = 5.1, 8.0$ Hz).

(24*R*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homostigmastane (41). Compound **41** was obtained from **38** as described above in the general method for the hydrogenation of the unsaturated i-methyl ethers. ¹H NMR (600 MHz): 3.328 (3H, s), 2.772 (1H, t, *J* = 2.7 Hz), 1.202 (3H, s), 1.204 (3H, s), 1.012 (3H, t, *J* = 7.4 Hz), 0.955 (3H, d, *J* = 6.6 Hz), 0.925 (3H, t, *J* = 7.4 Hz), 0.721 (3H, s), 0.650 (1H, dd, *J* = 4.1, 4.8 Hz), 0.434 (1H, dd, *J* = 5.1, 8.0 Hz).

(24*R*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homostigmastane (42). Compound **42** was obtained from **39** as described above in the general method for the hydrogenation of the unsaturated i-methyl ethers. ¹H NMR (600 MHz): 3.327 (3H, s), 2.772 (1H, t, *J* = 2.7 Hz), 1.206 (3H, s), 1.024 (3H, s), 0.978 (3H, t, *J* = 7.4 Hz), 0.974 (3H, d, *J* = 6.6 Hz), 0.951 (1H, t, *J* = 7.4 Hz), 0.718 (3H, s), 0.650 (1H, dd, *J* = 4.1, 5.0 Hz), 0.434 (1H, t, *J* = 5.1, 8.1 Hz).

(24*S*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homoergostane (62). Compound **62** was obtained from **58** as described above in the general method for the hydrogenation of the unsaturated i-methyl ethers. ¹H NMR (600 MHz): 3.325 (3H, s), 2.770 (1H, t, *J* = 2.7 Hz), 1.177 (3H, s), 1.168 (3H, s), 1.024 (3H, s), 1.016 (3H, t, *J* = 7.4 Hz), 0.926 (3H, d, *J* = 6.6 Hz), 0.721 (3H, s), 0.648 (1H, dd, *J* = 4.1, 4.7 Hz), 0.433 (1H, dd, *J* = 5.1, 8.0 Hz).

(24*R*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homoergostane (63). Compound **63** was obtained from **59** as described above in the general method for the hydrogenation of the unsaturated i-methyl ethers. ¹H NMR (600 MHz): 3.327 (3H, s), 2.772 (1H, t, *J* = 2.7 Hz), 1.175 (3H, s), 1.173 (3H, s), 1.031 (3H, t, *J* = 7.4 Hz), 1.024 (3H, s), 0.935 (3H, d, *J* = 6.6 Hz), 0.718 (3H, s), 0.650 (3H, dd, *J* = 4.1, 4.7 Hz), 0.434 (1H, dd, *J* = 5.1, 7.9 Hz).

(24*S*,25*S*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homoergostane (64). Compound **64** was obtained from **60** as described above in the general method for the hydrogenation of the unsaturated i-

methyl ethers. ^1H NMR (600 MHz): 3.326 (3H, s), 2.770 (1H, t, $J = 2.7$ Hz), 1.205 (3H, s), 1.195 (3H, s), 1.024 (3H, s), 0.974 (3H, t, $J = 7.4$ Hz), 0.926 (3H, d, $J = 6.6$ Hz), 0.722 (3H, s), 0.649 (1H, dd, $J = 4.0$, 4.8 Hz), 0.433 (1H, dd, $J = 5.0$, 7.9 Hz).

(24*R*,25*R*)-Dichloromethano-6 β -methoxy-3 α ,5 α -cyclo-26-homoergostane (65). Compound **65** was obtained from **61** as described above in the general method for the hydrogenation of the unsaturated i-methyl ethers. ^1H NMR (600 MHz): 3.327 (3H, s), 2.771 (1H, t, $J = 2.7$ Hz), 1.204 (3H, s), 1.194 (3H, s), 1.023 (3H, s), 0.975 (3H, t, $J = 7.4$ Hz), 0.930 (3H, d, $J = 6.6$ Hz), 0.716 (3H, s), 0.650 (1H, dd, $J = 4.1$, 4.7 Hz), 0.434 (1H, dd, $J = 5.1$, 7.9 Hz).

(24*S*)-Dichloromethano-6 β -Methoxy-3 α ,5-cyclo-26,27-dihomoergostane (85). Compound **85** was obtained from **81** and **83** as described above in the general method for the hydrogenation of the unsaturated i-methyl ethers. ^1H NMR (600 Hz): 3.325 (3H, s), 2.770 (1H, t, $J = 2.7$ Hz), 1.194 (3H, s), 1.023 (3H, s), 0.970 (3H, t, $J = 7.4$ Hz), 0.926 (3H, d, $J = 6.6$ Hz), 0.912 (1H, t, $J = 7.4$ Hz), 0.720 (3H, s), 0.650 (1H, dd, $J = 4.1$, 4.8 Hz), 0.433 (1H, dd, $J = 5.1$, 8.0 Hz).

(24*R*)-Dichloromethano-6 β -Methoxy-3 α ,5-cyclo-26,27-dihomoergostane (86). Compound **86** was obtained from **82** and **84** as described above in the general method for the hydrogenation of the unsaturated i-methyl ethers. ^1H NMR (600 MHz): 3.327 (3H, s), 2.771 (1H, t, $J = 2.7$ Hz), 1.195 (3H, s), 1.024 (3H, s), 0.983 (3H, t, $J = 7.4$ Hz), 0.934 (3H, d, $J = 6.6$ Hz), 0.908 (3H, t, $J = 7.4$ Hz), 0.717 (3H, s), 0.650 (1H, dd, $J = 4.1$, 4.8 Hz), 0.434 (1H, dd, $J = 5.1$, 8.0 Hz).

(24*S*,25*R*)-Dichloromethano-26-homostigmast-5-en-3 β -ol (43). Compound **43** was obtained from **40** as described above in the general method for the deprotection of the i-methyl ethers. ^1H NMR (600 MHz): 5.355 (1H, m), 3.524 (1H, m), 1.201 (3H, s), 1.011 (3H, s), 0.997 (3H, t, $J = 7.4$ Hz), 0.947 (3H, d, $J = 6.6$ Hz), 0.905 (3H, t, $J = 7.4$ Hz), 0.687 (3H, s).

(24R,25S)-Dichloromethano-26-homostigmast-5-en-3 β -ol (44). Compound **44** was obtained from **41** as described above in the general method for the deprotection of the i-methyl ethers. ^1H NMR (600 MHz): 5.354 (3H, m), 3.526 (1H, m), 1.202 (3H, s), 1.012 (3H, t, $J = 7.4$ Hz), 1.011 (3H, s), 0.961 (3H, d, $J = 6.6$ Hz), 0.925 (3H, t, $J = 7.4$ Hz), 0.685 (3H, s).

(24R,25R)-Dichloromethano-26-homostigmast-5-en-3 β -ol (45). Compound **45** was obtained from **42** as described above in the general method for the deprotection of the i-methyl ethers. ^1H NMR (600 MHz): 5.352 (1H, m), 3.525 (1H, m), 1.205 (3H, s), 1.010 (3H, s), 0.976 (3H, t, $J = 7.4$ Hz), 0.974 (3H, t, $J = 7.4$ Hz), 0.958 (3H, d, $J = 6.6$ Hz), 0.683 (3H, s).

(24S,25R)-Dichloromethano-6 β -26-homoergost-5-en-3 β -ol (66). Compound **66** was obtained from **62** as described above in the general method for the deprotection of the i-methyl ethers. ^1H NMR (600 MHz): 5.353 (1H, m), 3.524 (1H, m), 1.177 (3H, s), 1.167 (3H, s), 1.011 (3H, s), 1.016 (3H, t, $J = 7.4$ Hz), 0.932 (3H, d, $J = 6.6$ Hz), 0.686 (3H, s).

(24R,25S)-Dichloromethano-26-homoergost-5-en-3 β -ol (67). Compound **67** was obtained from **63** as described above in the general method for the deprotection of the i-methyl ethers. ^1H NMR (600 MHz): 5.353 (1H, m), 3.525 (1H, m), 1.174 (3H, s), 1.172 (3H, s), 1.010 (3H, s), 1.030 (3H, t, $J = 7.4$ Hz), 0.941 (3H, d, $J = 6.6$ Hz), 0.682 (3H, s).

(24S,25S)-Dichloromethano-26-homoergost-5-en-3 β -ol (68). Compound **68** was obtained from **64** as described above in the general method for the deprotection of the i-methyl ethers. ^1H NMR (600 MHz): 5.353 (1H, m), 3.524 (1H, m), 1.205 (3H, s), 1.194 (3H, s), 1.010 (3H, s), 0.972 (3H, t, $J = 7.4$ Hz), 0.933 (3H, d, $J = 6.6$ Hz), 0.686 (3H, s).

(24*R*,25*R*)-Dichloromethano-26-homoergost-5-en-3 β -ol (69). Compound **69** was obtained from **65** as described above in the general method for the deprotection of the i-methyl ethers. ¹H NMR (600 MHz): 5.353 (1H, m), 3.524 (1H, m), 1.202 (3H, s), 1.193 (3H, s), 1.010 (3H, s), 0.973 (3H, t, *J* = 7.4 Hz), 0.941 (3H, d, *J* = 6.6 Hz), 0.681 (3H, s).

(24*S*)-Dichloromethano-26,27-dihomoergost-5-en-3 β -ol (87). Compound **87** was obtained from **85** as described above in the general method for the deprotection of the i-methyl ethers. ¹H NMR (600 MHz): 5.354 (1H, m), 3.519 (1H, m), 1.193 (3H, s), 1.010 (3H, s), 0.970 (3H, t, *J* = 7.4 Hz), 0.932 (3H, d, *J* = 6.6 Hz), 0.910 (3H, t, *J* = 7.4 Hz), 0.685 (3H, s).

(24*R*)-Dichloromethano-26,27-dihomoergost-5-en-3 β -ol (88). Compound **88** was obtained from **86** as described above in the general method for the deprotection of the i-methyl ethers. ¹H NMR (600 MHz): 5.353 (1H, m), 3.524 (1H, m), 1.194 (3H, s), 1.010 (3H, s), 0.983 (3H, t, *J* = 7.4 Hz), 0.941 (3H, d, *J* = 6.6 Hz), 0.906 (3H, t, *J* = 7.4 Hz), 0.682 (3H, s).

23-[(1*R*,2*S*)-(1,2-Diethyl-2-methylcyclopropyl)-24-norchol-5-en-3 β -ol (46). Compound **46** was obtained from **43** as described above in the general method for the reduction of the dichlorocyclopropanes. ¹H NMR (800 MHz): 5.353 (1H, m), 3.523 (1H, m), 1.060 (3H, s), 1.010 (3H, s), 0.914 (3H, d, *J* = 6.6 Hz), 0.914 (3H, t, *J* = 7.4 Hz), 0.872 (3H, t, *J* = 7.4 Hz), 0.680 (3H, s), 0.047 (1H, d, *J* = 4.0 Hz), -0.004 (1H, t, *J* = 4.0 Hz).

23-[(1*S*,2*R*)-(1,2-Diethyl-2-methylcyclopropyl)-24-norchol-5-en-3 β -ol (47). Compound **47** was obtained from **44** as described above in the general method for the reduction of the dichlorocyclopropanes. ¹H NMR (800 MHz): 5.353 (1H, m), 3.523 (1H, m), 1.057 (3H, s), 1.011 (3H, s), 0.921 (3H, t, *J* = 7.4 Hz), 0.912 (3H, d, *J* = 6.6 Hz), 0.881 (3H, t, *J* = 7.4 Hz), 0.679 (3H, s), 0.020 (1H, d, *J* = 4.0 Hz), -0.012 (1H, d, *J* = 4.0 Hz).

23-[(1S,2S)-(1,2-Diethyl-2-methylcyclopropyl)]-24-norchol-5-en-3 β -ol (48). Compound **48** was obtained from **45** as described above in the general method for the reduction of the dichlorocyclopropanes. ¹H NMR (800 MHz): 5.353 (1H, m), 3.523 (1H, m), 1.069 (3H, s), 1.011 (3H, s), 0.922 (3H, t, *J* = 6.6 Hz), 0.893 (3H, t, *J* = 7.4 Hz), 0.886 (3H, t, *J* = 7.4 Hz), 0.679 (3H, s), 0.050 (1H, d, *J* = 4.0 Hz), -0.021 (1H, d, *J* = 4.0 Hz).

23-[(1R,2S)-(2-Ethyl-1,2-dimethylcyclopropyl)]-24-norchol-5-en-3 β -ol (1). Compound **1** was obtained from **66** as described above in the general method for the reduction of the dichlorocyclopropanes. ¹H NMR (800 MHz): 5.354 (1H, m), 3.523 (1H, m), 1.029 (3H, s), 1.029 (3H, s), 1.011 (3H, s), 0.919 (3H, t, *J* = 7.4 Hz), 0.905 (3H, d, *J* = 6.6 Hz), 0.680 (3H, s), 0.075 (1H, d, *J* = 4.0 Hz), -0.014 (1H, d, *J* = 4.0 Hz).

23-[(1S,2R)-(2-Ethyl-1,2-dimethylcyclopropyl)]-24-norchol-5-en-3 β -ol (70). Compound **70** was obtained from **67** as described above in the general method for the reduction of the dichlorocyclopropanes. ¹H NMR (800 MHz): 5.354 (1H, m), 3.524 (1H, m), 1.033 (3H, s), 1.030 (3H, s), 1.011 (3H, s), 0.927 (3H, t, *J* = 7.4 Hz), 0.903 (3H, d, *J* = 6.6 Hz), 0.677 (3H, s), 0.055 (1H, d, *J* = 4.0 Hz), -0.017 (3H, t, *J* = 4.0 Hz) .

23-[(1R,2R)-(2-Ethyl-1,2-dimethylcyclopropyl)]-24-norchol-5-en-3 β -ol (71). Compound **71** was obtained from **68** as described above in the general method for the reduction of the dichlorocyclopropanes. ¹H NMR (800 MHz): 5.353 (1H, m), 3.523 (1H, m), 1.053 (3H, s), 1.050 (3H, s), 1.011 (3H, s), 0.910 (3H, d, *J* = 6.6 Hz), 0.906 (3H, t, *J* = 7.4 Hz), 0.683 (3H, s), 0.062 (2H, s).

23-[(1S,2S)-(2-Ethyl-1,2-dimethylcyclopropyl)]-24-norchol-5-en-3 β -ol (72). Compound **72** was obtained from **69** as described above in the general method for the reduction of the dichlorocyclopropanes. ¹H NMR (800 MHz): 5.353 (1H, m), 3.524 (1H, m), 1.056 (3H, s), 1.049 (3H, s),

1.011 (3H, s), 0.911 (3H, d, $J = 6.6$ Hz), 0.909 (3H, t, $J = 7.4$ Hz), 0.679 (3H, s), 0.070 (1H, d, $J = 3.9$ Hz), 0.051 (1H, d, $J = 4.0$ Hz).

23-[(1*R*)-(2,2-Diethyl-1-methylcyclopropyl)]-24-norchol-5-en-3 β -ol (89). Compound **89** was obtained from **87** as described above in the general method for the reduction of the dichlorocyclopropanes. ^1H NMR (800 MHz): 5.353 (1H, m), 3.523 (1H, m), 1.061 (3H, s), 1.011 (3H, s), 0.906 (3H, d, $J = 6.6$ Hz), 0.883 (3H, t, $J = 7.4$ Hz), 0.864 (3H, d, $J = 7.4$ Hz), 0.681 (3H, s), 0.049 (1H, d, $J = 4.0$ Hz), -0.007 (1H, d, $J = 4.0$ Hz).

23-[(1*S*)-(2,2-Diethyl-1-methylcyclopropyl)]-24-norchol-5-en-3 β -ol (2). Compound **2** was obtained from **88** as described above in the general method for the reduction of the dichlorocyclopropanes. ^1H NMR (800 MHz): 5.353 (1H, m), 3.523 (1H, m), 1.061 (3H, s), 1.010 (3H, s), 0.905 (3H, d, $J = 6.6$ Hz), 0.889 (3H, t, $J = 7.4$ Hz), 0.864 (3H, t, $J = 7.4$ Hz), 0.677 (1H, s), 0.036 (1H, d, $J = 4.0$ Hz), -0.006 (1H, d, $J = 4.0$ Hz).

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CHAPTER 7

Modifications of the Kirk-Petrow Reaction: Use of a Sealed Reaction Vessel and an Odorless Thiol

Abstract

Improvements of the Kirk-Petrow reaction were made. The use of a sealed reaction vessel reduced the reaction time by at least a day with methanol as the solvent. The use of 1-dodecanethiol instead of thiophenol, the mercaptan normally used in the reaction, gave a relatively odorless reaction that proceeded in good yield and with equivalent reactivity to the typical Kirk-Petrow reaction product. Cholest-1-en-3-one was used as a substrate for the Kirk-Petrow reaction to probe its mechanism. These modifications of the Kirk-Petrow reaction make it a more appealing method for the selective alkylation of Δ^4 -3-oxo-steroids. Cholest-1-en-3-one was used as a substrate for the Kirk-Petrow reaction to probe its mechanism.

7.1 Introduction

The primary sterols in most dinoflagellates are 4-methyl sterols, and they are present in other organisms as biosynthetic intermediates of normal 4-desmethyl sterols.¹⁻³ The Kirk-Petrow reaction, a specific 4-thiomethylation of 3-oxo- Δ^4 -steroids, followed by subsequent desulfurization offers a facile

and effective route to 4 α -methyl sterols (Figure 7.1).^{4,5,7} Other reported methods to monomethylate at C-4 of 3-oxo- Δ^4 -steroids have been described as awkward in practice and unselective.^{4,6} The Kirk-Petrow reaction is an easy and useful method; however, the traditionally used mercaptan in the reaction, thiophenol, makes the workup and purification process of the crude product extremely unpleasant due to the foul odor of unreacted excess thiophenol. The desulfurization of the phenylthiomethyl products also produces an unpleasant odor from the regenerated thiophenol as a side product of the dissolving metal reduction (Li/NH₃). The reaction is also relatively slow as it requires days to reach completion when not done in triethanolamine as a polar basic catalyst and solvent.³ The addition of HCO₂K with n-PrOH as a solvent was also found to reduce the reaction time to a day.⁸ A modified version of the Kirk-Petrow reaction using a relatively odorless mercaptan, 1-dodecanethiol, is reported. The use of a sealed pressure vessel was found to reduce the reaction time by at least one day with methanol as a solvent. The Kirk-Petrow reaction was also carried out with cholest-1-en-3-one⁹ to probe the reaction mechanism.

7.2 Results and Discussion

To see if the Kirk-Petrow reaction could be improved, a series of solvents that included MeOH, EtOH and n-PrOH and THF were tested under reflux conditions with stigmast-22,4-dien-3-one as the starting 3-oxo- Δ^4 -steroid (Table 7.1). The reactions were worked up after 19 h to make sure they could be compared before reaching completion. THF was found to be the least efficient solvent with only 27% of desired product observed compared to starting material. MeOH and EtOH worked similarly well and were found to outperform n-PrOH. That result was a bit surprising since n-PrOH was expected to be the better solvent for this reaction because of its higher boiling point (97 °C), compared to 65 and 78 °C for MeOH and EtOH, respectively.

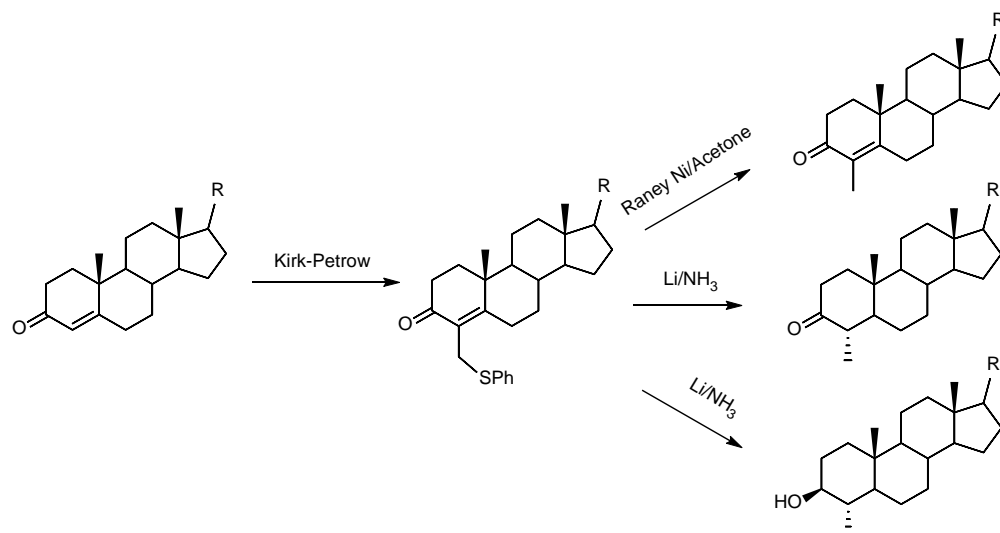


Figure 7.1. Possible 4-methyl sterols from the Kirk-Petrow reaction after desulfurization.

Table 7.1. Product (P) and starting material (SM) ratios of the Kirk-Petrow reaction in different solvents at reflux after 19 h.

	MeOH	EtOH	n-PrOH	THF
% SM	27	27	47	73
% P	73	73	53	27

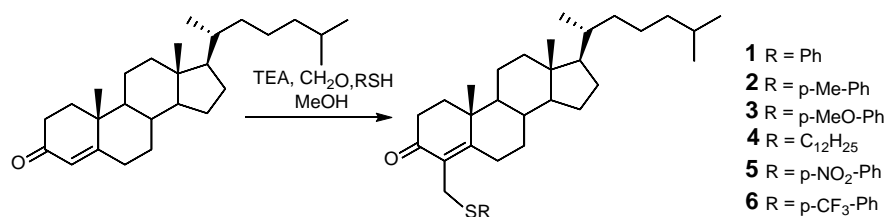
Another experiment was conducted to study the effect of cutting the amounts of thiol, TEA and formalin (37% formaldehyde in water) on the yield of Kirk-Petrow reaction. Since all reagents were in large excess in the general method, it made sense to determine if the excess reagents were necessary. A summary of the results is presented in Table 7.2. Using only 25 percent (3.4 eq.) of formalin did affect the reaction as only 18% of desired product observed after 19 h compared to 53% of product in the control reaction which contained 13.7 eq. of formalin. Reducing the amounts of TEA or thiophenol by 75% had relatively no effect on the reaction yield. Reducing the amounts of base and thiol by 75% simultaneously did not work as well. No significant difference in the amount of desired product was observed when the amount of the TEA was reduced to 25% (2.5 eq.). This observation made sense as TEA is only used as

catalytic base in the Kirk-Petrow reaction.³ However, reducing the TEA to 6.25 % led to a significantly worse reaction with only 11% of desired product after 19 h.

Table 7.2. Product (P) and starting material (SM) ratios of the Kirk-Petrow reaction with less thiol, formaldehyde and TEA. a) The control was set up in n-PrOH with 0.073 mmol of the starting enone, 1.00 mmol of formalin, 0.72 mmol of TEA and 1.47 mmol of thiophenol for 19 h at reflux.

	Control ^a	25% Thiol	25% CH ₂ O	25% TEA	12.5% TEA	12.5% Thiol	25% (Thiol and TEA)
% SM	47	46	82	45	89	86	65
% P	53	54	18	55	11	14	35

The Kirk-Petrow reaction was also set up with a series of substituted-phenylthiols, and 1-dodecanethiol to investigate their reactivities (Scheme 7.1). The reactions were done at reflux overnight and the crude products were analyzed by NMR (Table 7.3). The reaction with thiophenol gave the best results after 19 h with 51% product and 49% starting material. 4-Methylthiophenyl and 4-methoxythiophenol also worked relatively well with 48% and 46% of product, respectively. 1-Dodecanethiol only gave 30% of product under identical reaction conditions. The lowest product yields, 18% and 7%, were observed in reactions with 4-nitrothiophenol and 4-(trifluoromethyl)thiophenol, respectively. It is worth noting that those two thiols are substituted with electron withdrawing groups, however, it is unclear how that property affected their reactivities as the thiol with the 4-nitro substituent, the stronger electron withdrawing group, outperformed the one with 4-trifluoromethyl substituent.



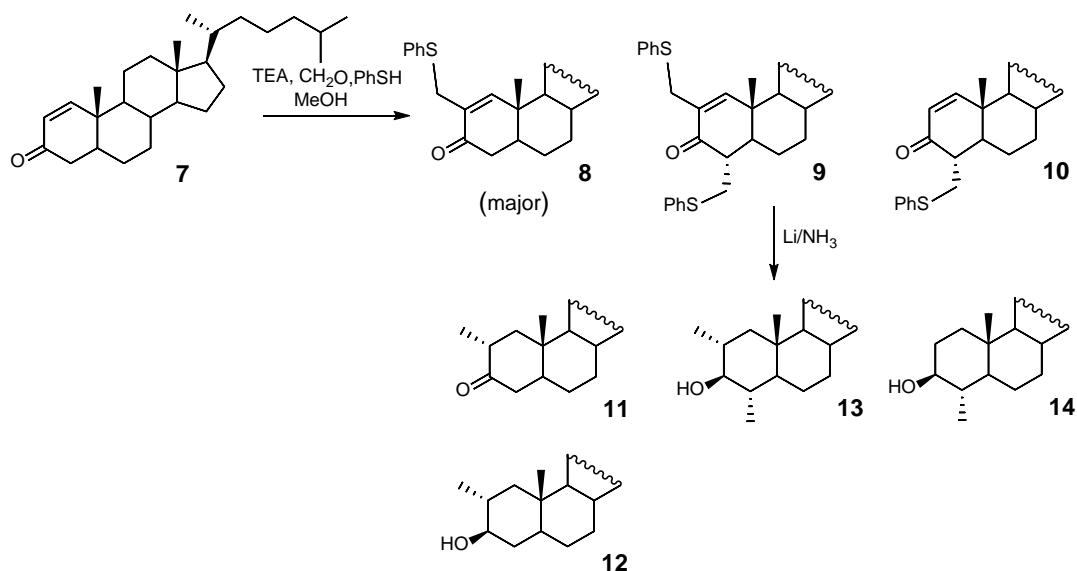
Scheme 7.1. Kirk-Petrow reaction with different thiols.

Table 7.3. Product (P) and starting material (SM) ratios of the Kirk-Petrow reaction with different thiols at reflux after 19 h.

Mercaptan	% SM	% Product
PhSH	49	51
p-Me-PhSH	52	48
p-MeO-PhSH	54	46
C ₁₂ H ₂₅ SH	70	30
p-NO ₂ -PhSH	82	18
p-CF ₃ -PhSH	93	7

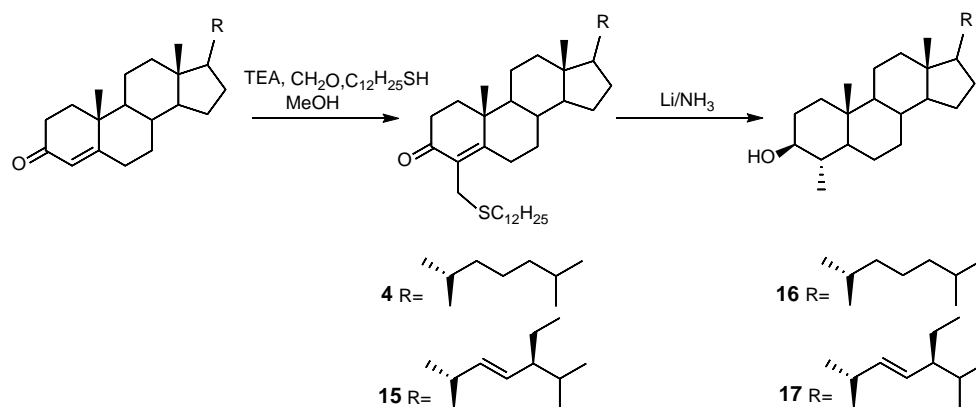
It was thought that evaporation of formaldehyde might adversely affect the Kirk-Petrow reaction. An attempt of the reaction in a sealed reaction pressure vessel showed an increased rate of product formation from about 53 to 90 % after 19 h with n-PrOH as the solvent. Product yields also increased to at least 90 % with MeOH and EtOH after 19 h at 120 °C. Reducing the large excess of formaldehyde negatively affected the rate of product formation (Table 7.2). Based on those observations, our standard method of the Kirk-Petrow reaction now used MeOH as solvent and the reaction was done in a sealed vessel with 10.0 eq. of thiol or mercaptan, 10.0 eq. 37% aqueous formaldehyde and 2.0 eq. TEA 120 °C for 36 h.

The Kirk-Petrow reaction has been used mainly to selectively phenylthiomethylate 3-oxo- Δ^4 -steroids at C4.³⁻⁶ Cholest-1-en-3-one was used as a substrate to help understand the mechanism of the reaction. The reaction was not as selective with that substrate, however, it produced predominantly compound **8** in a 71% yield by NMR. After ^1H and ^{13}C NMR characterization, the desulfurization and reduction of compound **8** using Li in liquid NH_3 ^{7c} gave compound **11**^{10,12} and **12**¹⁰⁻¹² to confirm its structure. COSY, HSQC and HMBC analyses were also used to characterize compound **8**. The ^1H signal at δ 6.680 (1H, s), assigned as the C1 hydrogen, had HMBC correlations with C2 (δ 132.3), C3 (δ 198.4), C5 (δ 44.2), C9 (δ 50.1) C19 (δ 12.8) and C28 (δ 33.3). The structures of compounds **9** and **10** were deduced from the products obtained from their dissolving metal reduction (Li/NH_3), compounds **13** and **14**¹², respectively. Integration of phenyl hydrogens between δ 7.450 and δ 7.241 in the ^1H NMR of compound **9** also suggested the presence of two phenylthiomethyl moieties in the molecule. The major product (**8**) from this reaction confirmed that the Kirk-Petrow reaction goes through a Baylis-Hillman type mechanism.¹³ Compound **10** is likely the normal enolization product.



Scheme 7.2. The Kirk-Petrow reaction of compound **7** followed by a Li/NH_3 reduction of its products.

A relatively odorless thiol, 1-dodecanethiol, was used in the Kirk-Petrow reaction as a substitute for thiophenol with cholest-4-en-3-one and stigmast-4,22-dien-3-one (Scheme 7.2). This thiol has been used previously in synthesis as a substitute for other foul-smelling thiols¹⁴. The reaction was done using the method described above for 92 h. A longer reaction time was required and expected, since 1-dodecanethiol reacted slower than thiophenol in that reaction (Table 7.3). An extra step not listed in Scheme 6.4 was required to isolate the desired product from the unreacted thiol and its side products by chromatography. Treating the crude product with 5% TFA converted the thiol impurities to less polar substances that could easily be separated from the desired product by flash chromatography. Compounds **4** and **15** were successfully made in a 74% yield using that method (Scheme 7.3). Both products were odorless and their treatment with Li/N₃ produced their corresponding 4 α -methyl products **16**¹² and **17**⁷ in yields comparable to the normal thiophenol Kirk-Petrow reaction products.



Scheme 7.3. Modified Kirk-Petrow reaction with 1-dodecanethiol followed by a Li/NH₃ reduction of the product.

7.3 Summary

The Kirk-Petrow reaction is a useful method to convert 3-oxo- Δ^4 -steroids to 4-methyl steroids. However, dealing with thiophenol, a foul-smelling mercaptan, makes the process a bit unappealing. The use of an odorless mercaptan, 1-dodecanethiol, instead of thiophenol provided odorless alkylthiomethyl steroids with comparable reactivities as the phenylthiomethyl products. The reaction time was also reduced by performing the reaction in a sealed vessel at 120 °C. The Kirk-Petrow reaction with cholest-1-en-3-one gave the 2-phenylthiomethyl product in 71% yield. Unlike with the 3-oxo- Δ^4 -steroids, two other minor side products were observed. However, the major product of the Kirk-Petrow reaction with the cholest-1-en-3-one confirmed that the reaction goes through a Baylis-Hillman type mechanism.

7.4 Experimental

General Procedures. NMR spectra were obtained in CDCl_3 on a Bruker 600 MHz spectrometer (^1H at 600 MHz and ^{13}C at 151 MHz). Preparative thin layer chromatography (TLC) purifications were done with Sorbent Technologies (SORBTECH) silica gel HL thin TLC plates (20 x 20 cm^2 , 250 μm thick with glass backed support). All solvents were from commercial sources.

Method for the normal Kirk-Petrow reaction in different solvents. To solutions of stigmast-4-dien-3-one (30.1 mg, 0.070 mmol) of 1 mL of solvent (EtOH, MeOH, n-PrOH and THF) in stretched glass 10-mL culture tubes was added 350 μL of a mixture of TEA (500 μL), 37% aqueous formaldehyde (500 μL) and thiophenol (750 μL). The tubes were flamed sealed and heated at 120 °C for 19 h. Each reaction was allowed to cool to room temperature before extraction with 9:1 hexanes/EtOAc. The combined organic layers for each reaction were washed with sodium bicarbonate and brine, dried over Na_2SO_4 and

concentrated under reduced pressure. A sample of each crude product was analyzed by ^1H NMR (Table 1).

Method for the Kirk-Petrow reaction in n-PrOH with different mercaptans. To stretched tubes containing 0.44 mmol of a mercaptan was added a 580 μL of a solution of cholest-4-en-3-one (210.1 mg, 0.540 mmol), TEA (280 μL , 2.00 mmol), 37% aqueous formaldehyde (280 μL , 2.80 mmol) in 3.5 mL of n-PrOH. The tubes were flamed sealed and heated at 120 $^\circ\text{C}$ for 19 h. Each reaction was allowed to cool to room temperature before extraction with 9:1 hexanes/EtOAc. The combined organic layers for each reaction were washed with sodium bicarbonate and brine, dried over Na_2SO_4 and concentrated under reduced pressure. A sample of each crude product was analyzed by ^1H NMR (Table 2).

4-Phenylthiomethyl-cholest-4-en-3-one (1). ^1H NMR (600 MHz): 3.874 (2H, s), 2.696 (1H, dt, $J = 3.3$, 14.7 Hz), 2.400 (1H, d, $J = 4.5\text{Hz}$), 2.384 (1H, d, $J = 4.5\text{Hz}$), 1.152 (3H, s), 0.906 (3H, d, $J = 6.6$ Hz), 0.868 (3H, d, $J = 6.6$ Hz), 0.864 (3H, d, $J = 6.6$ Hz), 0.697.

4-(p-Methyl-phenylthiomethyl)-cholest-4-en-3-one (2). ^1H NMR (600 MHz): 3.832 (1H, d, $J = 11.9$ Hz), 3.809 (1H, d, $J = 11.9$ Hz), 2.657 (1H, dt, $J = 3.0$, 14.7 Hz), 2.383 (2H, m), 2.311 (3H, s), 1.140 (3H, s), 0.908 (3H, d, $J = 6.6$ Hz), 0.870 (3H, d, $J = 6.6$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.695 (3H, s). ^{13}C NMR (151 MHz): 197.0, 167.9, 136.8, 132.1, 129.5, 128.5, 56.2, 55.9, 54.2, 42.4, 39.7, 39.5, 39.4, 36.1, 35.8, 35.1, 34.8, 33.7, 31.9, 29.6, 28.21, 28.16, 28.0, 24.1, 23.8, 22.8, 22.5, 21.1, 21.0, 18.6, 17.8, 12.0.

4-(p-Methoxy-phenylthiomethyl)-cholest-4-en-3-one (3). ^1H NMR (600 MHz): 3.785 (1H, d, $J = 12$ Hz), 3.735 (1H, d, $J = 12$ Hz), 2.597 (1H, dt, $J = 3.2$, 14.7 Hz), 2.368 (2H, m), 1.130 (3H, s), 0.906 (3H, d, $J = 6.6$ Hz), 0.869 (3H, d, $J = 6.6$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.690 (3H, s).

4-(1-Dodecanylthiomethyl)-cholest-4-en-3-one (4). See detailed procedure below. ^1H NMR (600 MHz): 3.489 (1H, d, $J = 12.2$ Hz), 3.430 (1H, d, $J = 12.2$ Hz), 2.802 (1H, dt, $J = 3.2, 14.7$ Hz), 2.503 (1H, d, $J = 7.4$ Hz), 2.490 (1H, d, $J = 7.4$ Hz), 1.179 (3H, s), 0.903 (3H, d, $J = 6.6$ Hz), 0.876 (3H, t, $J = 7.0$ Hz), 0.863 (3H, d, $J = 6.6$ Hz), 0.858 (3H, d, $J = 6.7$ Hz), 0.706 (3H, s). ^{13}C NMR (151 MHz): 197.4, 167.2, 129.9, 56.1, 55.9, 54.2, 42.4, 39.7, 39.5, 39.3, 36.1, 35.7, 35.3, 34.9, 33.7, 32.9, 32.2, 21.9, 29.8, 29.7, 29.63, 29.62, 29.6, 29.34, 29.3, 28.9, 28.23, 28.2, 28.0, 25.9, 24.1, 23.8, 22.8, 22.7, 22.5, 21.1, 18.6, 17.9, 14.1, 12.0.

4-(p-Nitro-phenylthiomethyl)-cholest-4-en-3-one (5). ^1H NMR (600 MHz): 4.050 (1H, d, $J = 11.0$ Hz), 3.969 (2H, d, $J = 11.0$ Hz), 2.765 (1H, dt, $J = 3.2, 14.6$ Hz), 2.442 (2H, m), 1.207 (3H, s), 0.912 (3H, d, $J = 6.5$ Hz), 0.869 (3H, d, $J = 6.6$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.715 (3H, s).

4-(p-Trifluoromethyl-phenylthiomethyl)-cholest-4-en-3-one (6). ^1H NMR (600 MHz): 3.971 (1H, d, $J = 11.2$ Hz), 3.915 (1H, d, $J = 11.2$ Hz), 2.732 (1H, dt, $J = 3.2, 14.7$ Hz), 2.417 (2H, m), 1.177 (3H, s), 0.909 (3H, d, $J = 6.6$ Hz), 0.868 (3H, d, $J = 6.6$ Hz), 0.864 (3H, d, $J = 6.6$ Hz), 0.705 (3H, s).

General method for the Kirk-Petrow reaction in sealed pressure vials. To a solution of 3-oxo- Δ^4 -steroid in MeOH in a pressure flask was added thiophenol (10.0 eq.), 37% aqueous formaldehyde (10.0 eq.) and TEA (2.0 eq.). The flask was capped, and the mixture was stirred at 120 °C (oil bath) for 36 h. The reaction was allowed to cool to rt before extraction with 9:1 hexanes/EtOAc. The combined organic layers were washed with sodium bicarbonate and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified via silica gel chromatography with 19:1 hexanes/EtOAc (72-88 % yield).

2-phenylthiomethyl-cholest-1-en-3-one (8). This compound was obtained as the major product (71% by NMR) from the method described above from cholest-1-en-3-one **7**. ^1H NMR (600 MHz): 6.680 (1H, s),

3.698 (1H, d, $J = 13.7$ Hz), 3.559 (1H, d, $J = 13.7$ Hz), 2.339 (1H, dd, $J = 14.3, 17.7$ Hz), 2.236 (1H, dd, $J = 4.2, 17.7$ Hz), 1.967 (1H, dt, $J = 3.3, 12.7$ Hz), 0.912 (3H, d, $J = 6.6$ Hz), 0.872 (3H, d, $J = 6.6$ Hz), 0.868 (3H, d, $J = 6.6$ Hz), 0.848 (3H, s), 0.648 (3H, s). ^{13}C NMR (151 MHz): 198.4, 155.6, 135.5, 132.3, 131.9, 128.8, 126.9, 56.4, 56.2, 50.1, 44.2, 42.7, 41.0, 39.7, 39.5, 39.0, 36.1, 35.8, 35.5, 33.3, 31.3, 28.2, 28.0, 27.3, 24.1, 23.8, 22.8, 22.5, 21.1, 18.7, 12.8, 12.1.

2,4 α -Diphenylthiomethyl-cholest-1-en-3-one (9). This compound was obtained as a minor product (10% by NMR) from the method described above from cholest-1-en-3-one **7**. ^1H NMR (600 MHz): 7.156 (1H, m), 3.505 (1H, m), 3.360 (1H, dd, $J = 5.6, 13.1$ Hz), 3.192 (1H, dd, $J = 3.2, 13.1$ Hz), 2.711 (1H, dd, $J = 4.3, 15.5$ Hz), 1.219 (3H, s), 0.920 (3H, d, $J = 6.5$ Hz), 0.869 (3H, d, $J = 6.6$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.694 (3H, s).

4 α -Phenylthiomethyl-cholest-1-en-3-one (10). This compound was obtained as a minor product (5% by NMR) from the method described above from cholest-1-en-3-one **7**. ^1H NMR (600 MHz): 7.168 (1H, m), 6.713 (1H, s), 3.667 (1H, d, $J = 14.0$), 3.618 (1H, d, $J = 14.0$), 3.574 (1H, dd, $J = 3.6, 12.5$ Hz), 3.301 (1H, dd, $J = 4.3, 12.5$ Hz), 0.910 (3H, d, $J = 6.6$ Hz), 0.874 (3H, d, $J = 6.6$ Hz), 0.869 (3H, d, $J = 6.6$ Hz), 0.854 (3H, s), 0.643 (3H, s).

4-(1-Dodecanylthiomethyl)-cholest-4-en-3-one (4). To a solution of cholest-4-en-3-one (10.1 g, 0.026 0 mol) in 150 mL of MeOH in a glass pressure flask was added thiophenol (25 mL, 0.24 mol), 37% aqueous formaldehyde (25 mL, 0.25 mol) and TEA (6 mL, 0.043 mol). The flask was capped, and the mixture was stirred and heated at 120 °C for 92 h. The reaction was allowed to cool to room temperature before extraction with 9:1 hexanes/EtOAc. The combined organic layers were washed with sodium bicarbonate and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The solid crude product (34.5 g) was dissolved 85 mL of 5% TFA in DCM and stirred at 45 °C for 18 h. The mixture was diluted with 100 mL of hexanes and the solvents were removed under reduced pressure. The crude

product was purified via silica gel chromatography to afford 11.7 g, 74% yield. The less polar impurities from the excess thiol were removed with hexanes. The desired product was eluted with 39:1 and 19:1 hexanes/EtOAc.

4-(1-Dodecanylthiomethyl)-stigmast-4,22-dien-3-one (15). This compound was obtained from the method described above from stigmast-4,22-dien-3-one. ¹H NMR (600 MHz): 5.148 (1H, dd, *J* = 8.7, 15.2 Hz), 5.027 (1H, dd, *J* = 8.7, 15.2 Hz), 3.493 (1H, d, *J* = 12.2 Hz), 3.435 (1H, d, *J* = 12.2 Hz), 2.807 (1H, dt, *J* = 3.2, 14.6 Hz), 2.509 (1H, d, *J* = 7.5 Hz), 2.496 (1H, d, *J* = 7.5 Hz), 1.186 (3H, s), 1.015 (3H, d, *J* = 6.6 Hz), 0.881 (3H, t, *J* = 7.0 Hz), 0.849 (3H, d, *J* = 6.5 Hz), 0.806 (3H, t, *J* = 7.3 Hz), 0.799 (3H, d, *J* = 6.6 Hz), 0.731 (3H, s). ¹³C NMR (151 MHz): 197.4, 167.2, 138.1, 129.9, 129.5, 56.0, 55.9, 54.3, 51.2, 42.3, 40.4, 39.6, 39.3, 35.3, 34.9, 33.7, 32.9, 32.2, 31.9, 31.85, 29.8, 29.7, 29.6, 29.56, 29.3, 29.27, 29.0, 28.9, 28.2, 25.9, 25.4, 24.2, 22.7, 21.1, 21.05, 19.0, 17.9, 14.1, 14.2, 12.2, 12.16.

General method for dissolving metal reduction of the Kirk-Petrow reaction products. To a solution of Li (approximately 60 mg, 8.8 mmol) of in 15 mL of NH₃ was added the 4-alkylthiomethyl- Δ^4 -3-oxo steroid (about 15 mg, 0.02 mmol) of in 2 mL of Et₂O. The mixture was stirred for 30 min before adding 400 μ L of MeOH dropwise. After 45 min, additional Li (35 mg, 5.1 mmol) was added to the reaction. After 1 h, NH₃ (5 mL) was added and the reaction was left to stir overnight. The residue obtained after the NH₃ slowly evaporated overnight was extracted with 4:1 hexanes/EtOAc and 10% hydrochloric acid. The combined organic layers were washed with sodium bicarbonate and brine, dried over Na₂SO₄ and concentrated under reduced pressure. The final product was purified via silica gel chromatography with 19:1-9:1 hexanes/EtOAc.

2 α -Methylcholestan-3-one (11). This compound was isolated as the major product of the dissolving metal reduction of the crude Kirk-Petrow reaction of cholest-1-en-3-one **10** as described above. ¹H NMR (600 MHz): 2.454 (1H, m), 2.310 (1H, t, *J* = 14.0 Hz), 1.056 (3H, s), 1.001 (3H, d, *J* = 6.6 Hz), 0.905

(3H, d, $J = 6.6$ Hz), 0.866 (3H, d, $J = 6.6$ Hz), 0.861 (3H, d, $J = 6.6$ Hz), 0.676 (3H, s). ^{13}C NMR (151 MHz): 213.2, 56.29, 56.27, 53.9, 48.6, 48.1, 44.8, 42.6, 41.2, 39.9, 39.5, 36.5, 36.2, 35.8, 35.2, 31.8, 28.8, 28.2, 28.0, 24.2, 23.8, 22.8, 22.6, 21.5, 18.7, 14.6, 12.4, 12.1.

2 α -Methylcholestan-3 β -ol (12). This compound was isolated as a minor product of the dissolving metal reduction of the crude Kirk-Petrow reaction of cholest-1-en-3-one **10** as described above. ^1H NMR (600 MHz): 3.132 (1H, m), 0.974 (3H, d, $J = 6.4$ Hz), 0.899 (3H, d, $J = 6.6$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.860 (3H, d, $J = 6.6$ Hz), 0.817 (3H, s), 0.647 (3H, s).

2 α ,4 α -Dimethylcholestan-3 β -ol (13). This compound was isolated as a minor product of the dissolving metal reduction of the crude Kirk-Petrow reaction of cholest-1-en-3-one **10** as described above. ^1H NMR (600 MHz): 2.632 (1H, t, $J = 9.9$ Hz), 0.982 (3H, d, $J = 6.5$ Hz), 0.952 (3H, d, $J = 6.5$ Hz), 0.898 (3H, d, $J = 6.6$ Hz), 0.864 (3H, d, $J = 6.6$ Hz), 0.861 (3H, d, $J = 6.6$ Hz), 0.834 (3H, s), 0.646 (3H, s).

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CHAPTER 8

Light Activated Phenyl Disulfide Oxidation of Allylic Alcohols via Thiyl Radicals

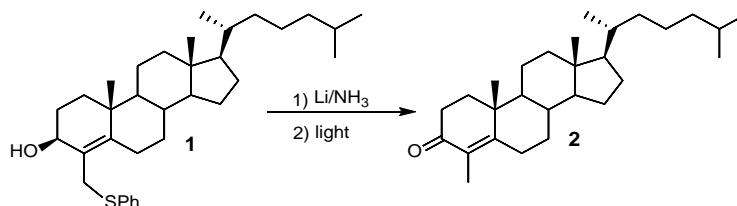
Abstract

A new method for the oxidation of allylic alcohols developed. A solution of phenyl disulfide in hexanes/EtOAc was found to oxidize allylic alcohols to their corresponding ketones and aldehydes under visible light. This new oxidation method was found to be selective and works very well for 3-hydroxy- Δ^4 -sterols. A few non-steroidal allylic and benzylic alcohols were also oxidized with the reaction in CDCl_3 as a solvent. This is the first report of the involvement of thiyl radicals in the oxidation of allylic alcohols.

8.1 Introduction

Numerous methods for the selective oxidation of allylic and benzylic alcohols to their respective α,β -unsaturated ketones and aldehydes have been reported.¹⁻¹² Those reactions often use metal based reagents that are either toxic, expensive or environmentally-unfriendly. Herein is described a new method that converts secondary allylic 3-hydroxy- Δ^4 -sterols into their respective conjugated enones in good yield using phenyl disulfide (Ph_2S_2) and visible light from 90 W tungsten lamp. This reaction was discovered

when the 3-oxo- Δ^4 -steroid (**2**)¹³ was mysteriously recovered as the only product from the hexanes/EtOAc extraction of the dissolving metal desulfurization of the phenylthiomethyl 3-hydroxy- Δ^4 -sterol **1** (Scheme 8.1). The crude extract containing compound **3** had been left uncapped under light over a weekend and compound **2** was obtained after removing the solvent under reduced pressure. Thiophenol and phenyl disulfide, two potential byproducts of the desulfurization reaction, were suspected to be involved in the observed oxidation reaction. Since the S-S bonds in disulfides are much easier to cleave photochemically than the S-H bonds of thiols,¹⁴ phenyl disulfide was used in a test and found to be the reagent responsible for the visible light activated allylic alcohol oxidation. The intermediates produced from the visible light-activated cleavage of the diphenyl sulfide are thiyl radicals, which have been well studied and extensively used in organic chemistry.¹⁴ However, their application in the oxidation of allylic alcohols has not been reported. This reaction was tested with different substrates and solvents to evaluate its potential as a viable general method for the selective oxidation of other allylic and benzylic alcohols.

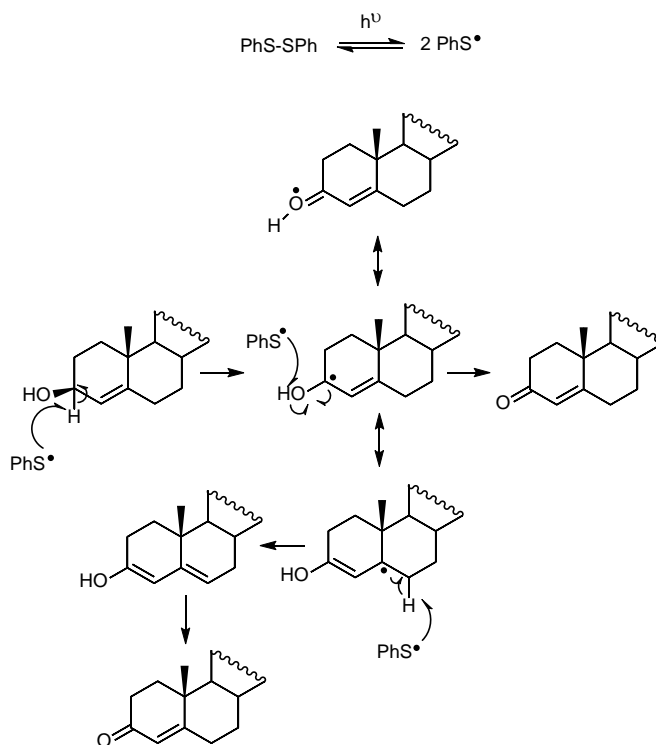


Scheme 8.1. Desulfurization and oxidation of compound **1**.

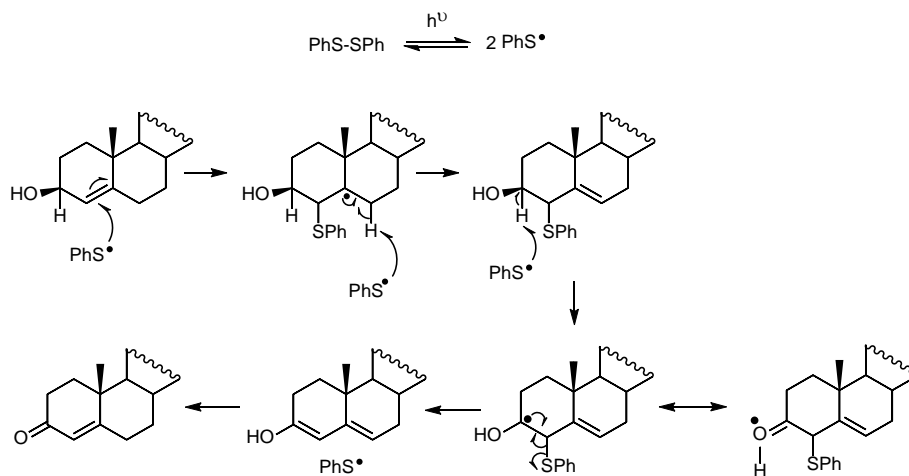
8.2 Results and Discussion

Stigmast-4,22-dien-3- β -ol (**8**), a steroidal allylic alcohol easily obtained from the commercially available ketone, was used as the substrate to test the reaction. Since the observed oxidation reaction occurred in a hexanes/EtOAc mixture, 9:1 hexanes/EtOAc reaction was used as the standard solvent. A mixture of hexanes/EtOAc has also been previously reported as one of the more efficient solvents for the

light activated reaction of 1-pentene, p-toluenethiol and oxygen to afford the corresponding β -hydroperoxy sulfide in good yield.¹⁵ As a test, a sample of a solution of compound **8** and Ph₂S₂ in 9:1 hexanes/EtOAc was left in a capped scintillation vial overnight under visible light while another sample of that same solution was left in another capped scintillation vial in dark. Compound **9** and some starting material were only observed in the scintillation vial that was left under the light and the vial that was left in the dark only contained starting material. The observation of product **9** only in the solution exposed to light confirmed that light is required for the oxidation to take place and that thiyl radicals were generated in situ during the reaction. Molecular oxygen maybe involved as an initiator in the formation of the thiyl radicals since this reaction is normally set up in the presence of air, which contains molecular oxygen. However, the oxidation product was still observed when the reaction was set up under a N₂ atmosphere. A proposed reaction mechanism is displayed in Scheme 8.2. This mechanism involves the formation of a resonance stabilized radical that leads to the enone and two molecules of thiophenol as a side product. Two possible paths to the enone from the original allylic radical are illustrated. The relatively stronger smell of thiophenol in the crude products confirmed its generation in the reaction. Another potential mechanism involving the addition of the thiyl radical to C-4 is described in Scheme 8.3. Thiyl radicals are known to quickly add to unsaturated systems via a reversible process.¹⁴ A phenylthiyl radical is regenerated to leave a dienol intermediate that ultimately tautomerizes to the desired conjugated enone. Two molecules of thiophenol are also produced according to this mechanism.



Scheme 8.2. Proposed mechanism for the allylic alcohol oxidation via a resonance stabilized radical when exposed to visible light.



Scheme 8.3. Proposed mechanism for the allylic alcohol oxidation involving a reversible thiyl radical addition.

A few solvents were tested with compound **8** to determine potential solvent effects (Table 8.1). Each reaction was set up with 5 eq. of Ph₂S₂ side-by-side in the standard reaction solvent 9:1 hexanes/EtOAc for 4 h. The use of less disulfide required a longer irradiation time and led to the formation of some side products. Out of the five tested solvents, hexanes and ethyl acetate gave the best results. No starting material was observed in any of the reactions, however, a dehydration product was present in all of them (Scheme 8.4). DCM and CDCl₃ gave the lowest yields by NMR and many unidentifiable side products.

Table 8.1. Product yields of oxidation reaction of compound **8** in a series of solvent.

Entry	Solvent	Yield (%)
1	Benzene	55
2	DCM	11
3	CDCl ₃	16
4	Hexanes	92
5	EtOAc	87
6	9:1 (Hexanes/EtOAc)	95

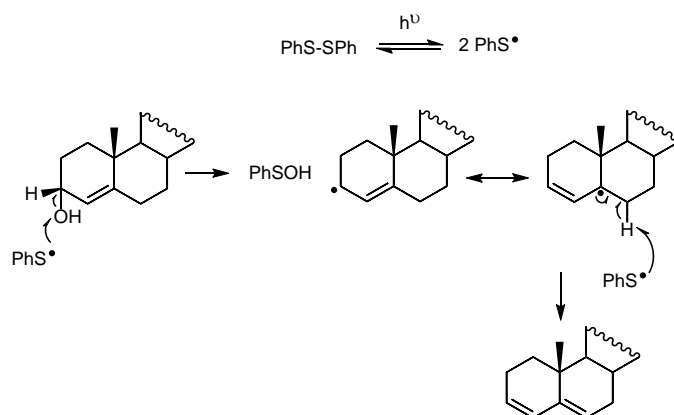
Thiophenol and di-tert-dodecyl disulfide were tested as potential thiyl radical sources in the oxidation reaction of compound **8** (Table 8.2). Since thiophenol, a foul-smelling thiol, is generated as a side product of this reaction with Ph₂S₂, di-tert-dodecyl disulfide was considered as an alternative source of thiyl radicals to keep the reaction odorless. Unfortunately, no reaction was observed with that disulfide. Since only starting material was observed, it is possible that the thiyl radicals were not generated under the reaction conditions or they self-quenched immediately to due instability. A 55% yield was observed with thiophenol compared to 95% yield with Ph₂S₂. The result with thiophenol was quite surprising as thiols normally require light less than 300 nm to generate thiyl radicals without an initiator.¹⁴ Since the oxidation reactions were done under air, molecular oxygen was likely the initiator involved in the

formation of the thiyl radicals in the reaction with thiophenol. Molecular oxygen has been reported as an initiator and co-initiator in many radical polymerization reactions.¹⁶ Molecular oxygen is also known to react with thiyl radicals form thiylperoxyl radicals (RSOO[•]) in a reversible process, a side reaction that may slow down the desired allylic oxidation.

Table 8.2. Oxidation reaction tests with different thiyl radical sources.

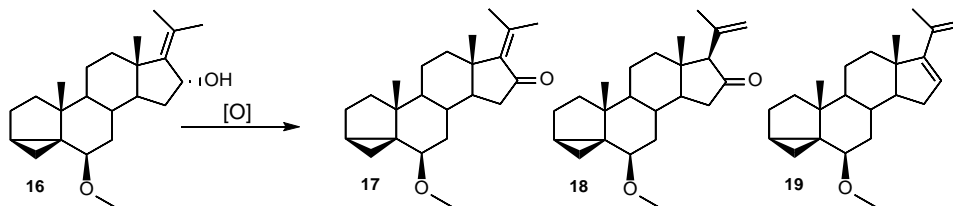
Entry	Thiyl Radical Source	Yield (%)
1	PhSH	55
2	Ph ₂ S ₂	95
3	(tert-Dodecyl) ₂ S ₂	0

This light activated oxidation method with phenyl disulfide was also found to be chemoselective as the oxidation was only observed with allylic systems; no reaction was observed with cholesterol and cholestanol. A series of steroidal substrates containing allylic alcohol were used to investigate the application of the reaction as viable oxidation method (Table 8.3). The reaction clearly worked better for entries 1, 2 and 4 to give their corresponding enones. It appears that the stereochemistry at the C-3 in the 3-hydroxy- Δ^4 -sterols influenced the yield of the oxidation reaction. A dehydration reaction that accounted for about a third of the product mixture was observed for compound **7** of the third entry and sigmast-4,22-dien-3- α -ol. A potential mechanism describing the dehydration reaction is described in Scheme 8.4. The 3-axial OH, which is sterically more available, is abstracted by the thiyl radical to form phenylsulfenic acid¹⁷ and leave behind the stable allylic radical that ultimately leads to the diene¹⁸. The same reactivity trend was observed for compounds **10**¹⁹ and **12**²⁰ to give the Δ^1 -enone **11**²¹.



Scheme 8.4. Proposed mechanism of the observed dehydration products.

The reaction did not work as well with all steroidal substrates. The nuclear homoallylic alcohols of compounds **13** and **15** were not oxidized as expected; however, the side chain allylic alcohols only produced a 1:1 mixture of the E/Z aldehydes in approximately 20%. Some dehydration products of the side chain hydroxyl were also observed in the crude reaction product. Compound **16**, a cyclic secondary allylic alcohol with an i-methyl nucleus, was oxidized very poorly via this method. About 1% of the desired product **17** was observed by ^1H NMR analysis of the crude reaction product. Overall, the reaction was extremely messy as the i-methyl nucleus was sensitive to the reaction conditions. To identify the desired product, compound **16** was oxidized with PCC and MnO_2 . Both oxidants gave a mixture of products that included compound **17**, the unconjugated enone **18** (Δ^{20} -16-keto-i-methyl ether) a dehydration product **19** ($\Delta^{16,20}$ -i-methyl ether) (Scheme 8.5).



Scheme 8.5. Oxidation of compound **16** with PCC and MnO_2 .

Table 8.3. Oxidation reaction of steroidal allylic alcohols.

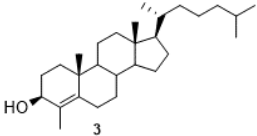
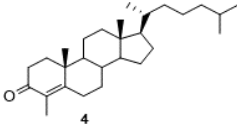
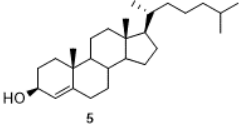
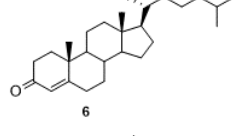
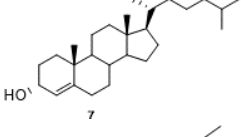
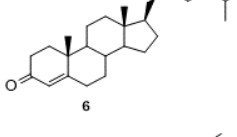
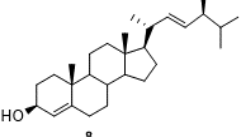
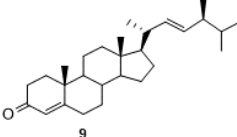
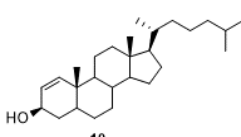
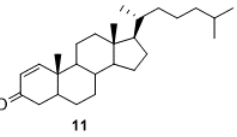
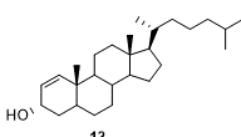
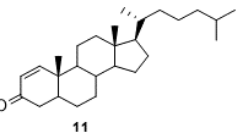
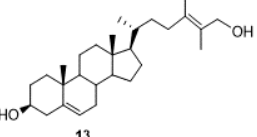
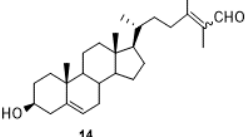
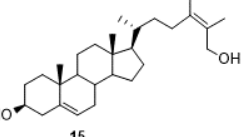
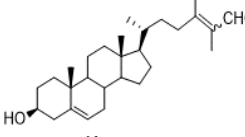
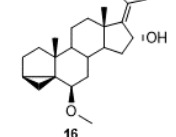
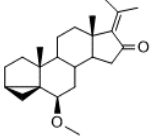




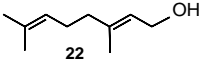
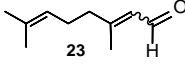
Entry	Substrate	Product	Yield (%)
1			99
2			97
3			65
4			95
5			60
6			12
7			20 (1:1 E/Z)
8			17 (1:1 E/Z)
9			>1

Table 8.4. Oxidation of non-steroidal substrates in CDCl₃ in sealed NMR tubes.

Entry	Substrate	Reaction time (h)	Product	Yield (%)
1	 18	22	 19	3
2	 20	4	 21	8
3	 22	24	 23	9 (2:1 E/Z)

The oxidation reaction was also attempted with some non-steroidal allylic and benzylic alcohols (Table 8.4). The reactions were done in CDCl₃ in flame sealed NMR tubes to assure a fairly accurate measurement of the yields by ¹H NMR. The standard solvent mixture, 9:1 hexanes/EtOAc, could not be used with those reactions because of the volatility of the starting materials and potential products and because of the NMR analyses. Overall, this oxidation method did not work well with those substrates as the observed yields based on product and starting material ratios were under 10 percent. The formation of the desired product for those compounds was much slower as mainly starting material was observed for all the reactions after 4 h. The poor yields could be associated with the solvent used in the reactions, CDCl₃, which was a relatively poor solvent in the oxidation of compound **8** (Table 8.1). Repeating those reactions on a larger scale and in the standard solvent should offer a better idea of the efficiency or limitation of that oxidation method. A signal observed at δ 3.444 in the crude products confirmed the production of thiophenol during the reactions as described in the proposed mechanisms. The formation of compound **19** during the oxidation of compound **18** was verified by the presence of the methyl ketone signal at δ 2.609 (3H, s) in the ¹H NMR of the crude reaction.²² Some side products and compound **21**²³ were observed in the oxidation of compound **20** after 4 h. Compound **23**, a 2:1 mixture of citral/neral ,

and a 2:1 mixture of compound **22**/nerol were observed from the oxidation of compound **22** after 24 h.²⁴ The presence of nerol and neral in the oxidation products is evidence of thiyl radical isomerization of the starting material, compound **22**.

8.3 Conclusions

A new and selective method of oxidation of allylic and benzylic alcohols with phenyl disulfide under visible light was described. Based on the tested substrates, this reaction appeared to be limited as a good method for the oxidation of 3-hydroxy- Δ^4 -sterols. 3 β -Hydroxy- Δ^4 -sterols were converted to their corresponding enones in near quantitative yield. Lower yields were obtained with 3 α -hydroxy- Δ^4 -sterols as a competing dehydration reaction was observed.

This reaction provides an extraction free method for high molecular weight starting materials as the products can easily be isolated via flash chromatography after the evaporation of the solvent. This method also provides the first account of the use of thiyl radicals in the selective oxidation of allylic alcohols. The thiyl radicals involved in the reaction are generated from the irradiation of phenyl disulfide with visible light from a 90W tungsten lamp without a photocatalyst. A recent study reported the use of phenyl disulfide with visible light as a source of thiyl radicals in an anti-Markovnikov hydration of olefins, however a photocatalyst was used.²⁵ It is unclear if molecular oxygen is involved in the generation of the thiyl radical in this reaction. The desired reaction product was observed when the reaction was set up under a nitrogen atmosphere. If molecular oxygen is important, it is more likely involved in the reaction in its triplet state, in which it can reversibly add to thiyl radicals to generate thiylperoxyl radicals. Singlet oxygen, which normally requires a sensitizer to be generated,²⁶ is unlikely to be involved as an initiator in this oxidation reaction.

8.4 Experimental

General Procedures. NMR spectra were obtained in CDCl_3 on a Bruker 600 MHz spectrometer (^1H at 600 MHz and ^{13}C at 151 MHz). A 90 W tungsten lamp was used as the source of light for the reactions. The lamp was placed 12 cm away at a 45-degree angle above the reaction vessels. Preparative thin layer chromatography (TLC) purifications were done with Sorbent Technologies (SORBTECH) silica gel HL thin TLC plates (20 x 20 cm^2 , 250 μm thick with glass backed support). All solvents were from commercial sources.

4-Phenylthiomethyl-cholest-4-en-3 β -ol (1). To a solution NaBH_4 (250.1 mg, 6.60 mmol) in 10.0 mL of MeOH at $-78\text{ }^\circ\text{C}$ (dry-ice acetone bath) was added 4-phenylthiomethyl-cholest-4-en-3-one (1.01 g, 2.0 mmol) in 10.0 mL of MeOH. The mixture was left to react overnight. The reaction was extracted with 10% aqueous HCl and 2:1 hexanes/EtOAc. The combined organic layers were washed with sodium bicarbonate and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified via silica gel chromatography with 19:1-4:1 hexanes/EtOAc to afford compound **1** (761.6 mg, 75% yield). ^1H NMR (600 MHz): 7.372 (2H, m), 7.270 (2H, t, $J = 7.5$ Hz), 7.183 (1H, m), 4.277 (1H, dd, $J = 6.8, 13.4$ Hz), 3.811 (1H, d, $J = 11.7$ Hz), 3.772 (1H, d, $J = 11.7$ Hz), 2.464 (1H, dt, $J = 3.3, 14.1$ Hz), 1.040 (3H, s), 0.901 (3H, d, $J = 6.6$ Hz), 0.870 (3H, d, $J = 6.6$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.666 (3H, s). ^{13}C NMR (151 MHz): 147.3, 136.9, 130.3, 128.8, 126.2, 125.7, 69.1, 56.2, 56.1, 53.7, 53.7, 42.5, 39.9, 39.5, 38.6, 36.1, 35.8, 35.5, 33.7, 33.2, 32.8, 29.0, 28.2, 28.0, 26.3, 24.1, 23.9, 22.8, 22.5, 21.3, 20.1, 18.6, 12.0.

General method for the light-activated Ph_2S_2 oxidation of the steroidal allylic alcohols. A solution the steroidal allylic alcohol (2-5 mg) and Ph_2S_2 (5.0 eq.) in 1.5 mL of 9:1 hexanes/EtOAc was irradiated with 90W tungsten lamp for 4 h. The reaction was evaporated under a flow of air without heat and the crude

product was analyzed by ^1H NMR. For the reactions in different solvents, 1.5 mL of each of the tested solvents were used instead of 1.5 mL of the standard 9:1 hexanes/EtOAc.

Cholest-1-en-3 β -ol (10) and cholest-1-en-3 α -ol (12). To a solution of compound **11** (5.2 mg, 0.014 mmol) in 3.0 mL of Et_2O was added 8.5 mg of LAH. The mixture was stirred at rt for 1 h before extraction with 10% hydrochloric acid and 2:1 hexanes/EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and filtered through a small pad of silica gel. The crude product of 10:1 of **10/12** was isolated as a white solid after removing the solvent mixture under a flow of N_2 and separated by preparative TLC with 4:1 hexanes/EtOAc to provide the compounds **12** (0.5 mg) (less polar product) and **10** (4.4 mg). **Cholest-1-en-3 β -ol (10).** ^1H NMR (600 MHz): 5.196 (1H, dd, $J = 1.6, 10.2$ Hz), 5.482 (1H, m), 4.302 (1H, m), 0.905 (3H, s), 0.902 (3H, d, $J = 6.6$ Hz), 0.865 (3H, d, $J = 6.6$ Hz), 0.860 (3H, d, $J = 6.6$ Hz), 0.663 (3H, s). **Cholest-1-en-3 α -ol (12).** ^1H NMR (600 MHz): 6.078 (1H, d, $J = 10.0$ Hz), 5.644 (1H, ddd, $J = 1.2, 4.4, 10.0$ Hz), 4.091 (1H, br s), 0.9 (3H, d, $J = 6.6$ Hz), 0.867 (3H, d, $J = 6.6$ Hz), 0.862 (3H, d, $J = 6.6$ Hz), 0.798 (3H, s), 0.669 (3H, s).

(24E)-Ergost-5,24-dien-3 β ,26-diol (13). This compound was obtained from the deprotection of its corresponding i-methyl sterol (see Chapter 6, compound **51b**). ^1H NMR (600 MHz): 5.352 (3H, m), 4.117 (2H, br s), 3.524 (1H, m), 1.747 (3H, m), 1.715 (3H, m), 1.010, 0.975 (3H, d, $J = 6.5$ Hz), 0.687 (3H, s).

(24Z)-Ergost-5,24-dien-3 β ,26-diol (15). This compound was obtained from the deprotection of its corresponding i-methyl sterol (see Chapter 6 compound **51a**). ^1H NMR (600 MHz): 5.352 (3H, m), 4.107 (2H, s), 3.524 (1H, m), 1.739 (3H, s), 1.669 (3H, s), 1.010 (3H, s), 0.965 (3H, d, $J = 6.5$ Hz), 0.680 (3H, s).

(24 ξ)-Ergost-5,24-dien-3 β -OH-26-al (14). Compound **11** was observed as a 1:1 mixture from the light-activated Ph_2S_2 oxidation of compounds **10** and **12** in 17% and 20% yields by NMR, respectively. The

products were not isolated from the crude reaction product. ^1H NMR (600 MHz): 10.135 (1H, s) and 10.103 (1H, s).

Compounds 17, 18 and 19. Those compounds were obtained as a mixture from the PCC and MnO_2 oxidations of compound **16**. For the PCC reaction, 5.2 mg of PCC was added to a solution **16** (2.1 mg, 6.1 μmol) in 1.5 mL of DCM. The reaction was stirred for 1 h. Hexanes (3 mL) was added to the mixture before filtering it through silica gel. The product mixture was eluted with 4:1 hexanes/EtOAc. For MnO_2 oxidation, 8.3 mg of MnO_2 were added to a solution of **16** (2.5 mg, 7.3 μmol) in 1.5 mL of DCM. The mixture was stirred for 16 h before filtering it through silica gel. The product mixture was eluted with 4:1 hexanes/EtOAc. The crude products for both reactions were purified via preparative TLC with 19:1 hexanes/EtOAc. Compound **19** was isolated pure while **17** and **18** were not separable by that method.

Compound 16: ^1H NMR (600 MHz): 4.635 (1H, d, $J = 5.1$ Hz), 3.343 (3H, s), 2.807 (1H, t, $J = 2.8$ Hz), 1.819 (3H, s), 1.759 (3H, s), 1.039 (3H, s), 0.903 (3H, s), 0.665 (1H, m), 0.452 (1H, dd, $J = 5.1, 8.0$ Hz).

Compound 17: ^1H NMR (600 MHz): 3.343 (3H, s), 2.806 (1H, t, $J = 2.8$ Hz), 2.184 (3H, s), 1.903 (3H, s), 1.074 (3H, s), 1.059 (3H, s), 0.680 (1H, m), 0.472 (1H, dd, $J = 5.1, 8.0$ Hz).

Compound 18: ^1H NMR (600 MHz): 5.076 (1H, m), 4.724 (1H, m), 3.347 (3H, s), 2.812 (1H, t, $J = 2.8$ Hz), 2.546 (1H, s), 1.772 (3H, br s), 1.068 (3H, s), 0.827 (3H, s), 0.695 (1H, m), 0.487 (1H, dd, $J = 5.1, 8.1$ Hz).

Compound 19: ^1H NMR (600 MHz): 5.727 (1H, m), 5.067 (1H, m), 4.859 (1H, m), 3.355 (3H, s), 2.805 (1H, t, $J = 2.8$ Hz), 1.878 (3H, s), 1.063 (3H, s), 1.000 (3H, s), 0.671 (1H, m), 0.454 (1H, dd, $J = 5.1, 8.1$ Hz).

General method for the light-activated Ph₂S₂ oxidation of the non-steroidal alcohols. A solution the steroidal allylic alcohol (2-5 mg) and Ph₂S₂ (5.0 eq.) in 500 µL of CDCl₃ in a flame sealed NMR tube was irradiated with a 90W tungsten lamp for 4-24 h. The reaction was monitored over time by ¹H NMR.

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Conclusions

The primary goal of this research was to synthesize unusual marine sterols and analogs for biological studies of their effects on ecologically important invertebrates like copepods. Unusual marine sterols are hypothesized to be present in some marine organisms as chemical defense against predation by invertebrates as they may interfere with the invertebrate phytosterol dealkylation pathway.¹ Marine invertebrates are unable to biosynthesize sterols and depend on their diet as a source of cholesterol, an essential component for somatic and reproductive growth.^{2,3} Pfiesterol and the simple analogs of petrosterol were synthesized to study their effects on phytosterol metabolism and the life history of some marine invertebrates. Two synthetic 24-ethylpavlovols and 4-methylcholestane-3 β ,4 β -diol were synthesized to generate samples to study their effects on bay scallop larvae. Natural pavlovols, unusual marine 3 β ,4 β -dihydroxy-4-methylsterols, are reported to cause early metamorphosis and early settlement induction in northern bay scallop (*Argopecten irradians irradians*) larvae.⁴ The 24-epimer of two unusual bioactive marine sponge sterols were synthesized to verify the stereochemistry of the natural sterols and obtain samples for bioactivity studies. The natural marine sponge sterols, theonellasterol and conicasterol, and some of their natural analogs are natural ligands of the human nuclear receptors pregnane X receptor (PXR) and farnesoid X receptor (FXR).⁵ Two unusual 24,25-cyclopropyl sterols from a tropical jewel orchid were also synthesized and identified through a series of chemical correlations ultimately resting on (24R)-24-ethyl-24-methylcholestanol⁶, the structure of which rests on crystallography. Two methods for sterol synthesis were also developed, a modification of the Kirk-Petrow reaction and a novel method to selectively oxidize allylic alcohols via visible light activated thiyl radicals.

The synthesis of 24-epiconicasterol was completed from ergosterol in a 10-step sequence and a 13% overall yield. The ¹H and ¹³C NMR data for 24-epiconicasterol were used to confirm the reported stereochemistry at C24 (24R) for the natural product conicasterol⁷. Substantial differences were observed

between the side chain ^1H and ^{13}C NMR signals of conicasterol and 24-epiconicaterol. To confirm the stereochemistry reported for theonellasterol⁷, correlations of the $\Delta\delta$ values between C29 and other side chain ^1H NMR signals of 24-epitheonellasterol and theonellasterol, and 24S and 24R 24-ethylcholesterols were used. Samples for biological studies were also obtained at the end of the synthesis. Bioactivity of the synthetic epimers would be of great significance as they could be an alternative source of ligands with pharmaceutical interests for the nuclear receptors PXR and FXR.

Pfiesterol, a potential biomarker of the toxic dinoflagellate *Pfiesteria piscicida*, was successfully synthesized from 16-dehydroprenolone acetate. Two different methods were used to generate the 4 α -methyl nucleus from the Δ^4 -enone intermediate **18b** (Chapter 3). The structure of the synthesized pfiesterol was confirmed by ^1H NMR comparison with the natural product and a sample previously prepared in our lab.⁸ The side chain of pfiesterol is unstable when stored in the freezer in CDCl_3 and the decomposition product was identified as 4-methylandrosterone,⁸ an androgenic steroid hormone that may negatively affect reproduction in invertebrates.⁹ A sample of pfiesterol stored in the freezer in benzene for over 2.5 years was found unchanged by ^1H NMR analysis. Pfiesterol was synthesized via a reductive methylation using $^{13}\text{CH}_3\text{I}$ to introduce a ^{13}C label to facilitate a positive identification of the decomposition product in biological experiments in invertebrates. Introducing the label late in the synthesis provided an economic method to make the labeled product, but the reductive methylation was a challenge because the intermediate enolate was extremely sensitive to moisture.

The synthesis of the 4-methylcholestane-3 β ,4 β -diol, a pavlovol, was accomplished from cholestenone in seven steps with a 38% overall yield. Its structure was confirmed by comparison of its ^1H and ^{13}C NMR data to that of the natural pavlovols 24S-methylpavlovol and 24S-ethylpavlovol.¹⁰ Another seven-step sequence was used to make 4-methylcholestane-3 β ,4 β -diol from 4-methylenecholestanol. The

second sequence was less effective as only an 8% overall yield was observed. Two new synthetic pavlovols, (24R)-4-methylstigmastane-3 β ,4 β -diol and (24R)-4-methylstigmast-22-en-3 β ,4 β -diol, were made from stigmasta-5,22-dien-3-one using the first method. All three pavlovols were made available for studies of their biological effects on bivalve growth and development. Natural pavlovols behave as analogs of ecdysone, a natural invertebrate hormone that is also involved in life-history transitions in bivalve molluscs.⁴

Two simple analogs of petrosterol, (24R)-24-cyclopropyl-24-methylchol-5-en-3 β -ol and (24S)-24-cyclopropyl-24-methylchol-5-en-3 β -ol, were successfully synthesized as a mixture from the i-methyl-22-iodide in six steps with a 43% overall yield. Since the purpose of the sequence was to synthesize those compounds as potential inhibitors of invertebrate sterol metabolism, producing them as a mixture was not an issue. The possible enzymatic products of the simple analogs of petrosterol, 24-cyclopropyl-24-methylenechol-5-en-3 β -ol and (24R)-epoxy-24-cyclopropyl-24-homochol-5-en-3 β -ol and (24S)-24,25-epoxy-24-cyclopropyl-24-homochol-5-en-3 β -ol, were also synthesized in good yield. Feeding experiments confirmed the inhibitory effects of these simple analogs of petrosterol and of the possible dehydrogenation product on invertebrate phytosterol metabolism.

Two unusual cyclopropyl sterols from the tropical jewel orchid were synthesized and identified as 23-[(1R,2S)-(2-ethyl-1,2-dimethylcyclopropyl)]-24-norchol-5-en-3 β -ol and 23-[(1S)-(2,2-diethyl-1-methylcyclopropyl)]-24-norchol-5-en-3 β -ol. Both unusual phytosterols and all their possible isomers were synthesized and characterized by 800 MHz ¹H NMR as only minute amounts of the natural sterols were isolated. Access to an 800 MHz NMR was instrumental to the process of discovering and ultimately characterizing those sterols. To the best of our knowledge, this is the first report of the isolation and characterization of sterols with a side chain cyclopropane in higher plants. The presence of those unusual

sterols in the aerial parts of those plants could be as chemical defense against herbivorous insects. Those complex sterols may impede the dealkylation process used by insects to convert phytosterols to cholesterol, an essential component for growth and reproduction.^{2,3} Feeding experiments with those sterols are necessary to verify this hypothesis.

The Kirk-Petrow reaction, a selective method to convert 3-oxo- Δ^4 -steroids to 4-methyl steroids^{11,12}, was modified to significantly shorten the reaction time by using a sealed glass pressure vessel. This modification helped by eliminating the loss of formaldehyde during the reaction which negatively affects the rate of product formation. To eliminate the inconvenience of working with thiophenol, a foul smelling mercaptan typically used in the Kirk-Petrow reaction, 1-dodecanethiol was used to obtain odorless alkylthiomethyl steroids with comparable reactivities as the phenylthiomethyl products. To probe the mechanism of the Kirk-Petrow reaction, cholest-1-en-3-one was used to obtain the 2-phenylthiomethyl product as the major product. Unlike with the 3-oxo- Δ^4 -steroids, two other minor side products were observed. The major product of the Kirk-Petrow reaction with the cholest-1-en-3-one confirmed that the reaction went through a Baylis-Hillman type mechanism.

A new and selective method of oxidation of allylic alcohols with phenyl disulfide under visible light was discovered and explored. Based on the tested substrates, this reaction appeared to be limited to the oxidation of 3-hydroxy- Δ^4 -sterols, which were converted to their corresponding enones in near quantitative yield. Lower yields (around 60%) were obtained with 3 α -hydroxy- Δ^4 -sterols where a competing dehydration reaction was observed. This reaction provides useful free method for high molecular weight starting materials as the products can easily be isolated via flash chromatography after the evaporation of the solvent. This method also features the first account of the use of thiyl radicals in the selective oxidation of allylic alcohols. The thiyl radicals involved in the reaction are generated from the

irradiation of phenyl disulfide with visible light from a 90W tungsten lamp without a photocatalyst. A recent study reported the use of phenyl disulfide with visible light as a source of thiyl radicals in an anti-Markovnikov hydration of olefins, however a photocatalyst was necessary to generate the thiyl radicals.¹³ If molecular oxygen is important, it is more likely involved in the reaction in its triplet state, in which it can reversibly add to thiyl radicals to generate thiylperoxyl radicals. Singlet oxygen, which normally requires a sensitizer to be generated,¹⁴ is unlikely to be involved as an initiator in this oxidation reaction.

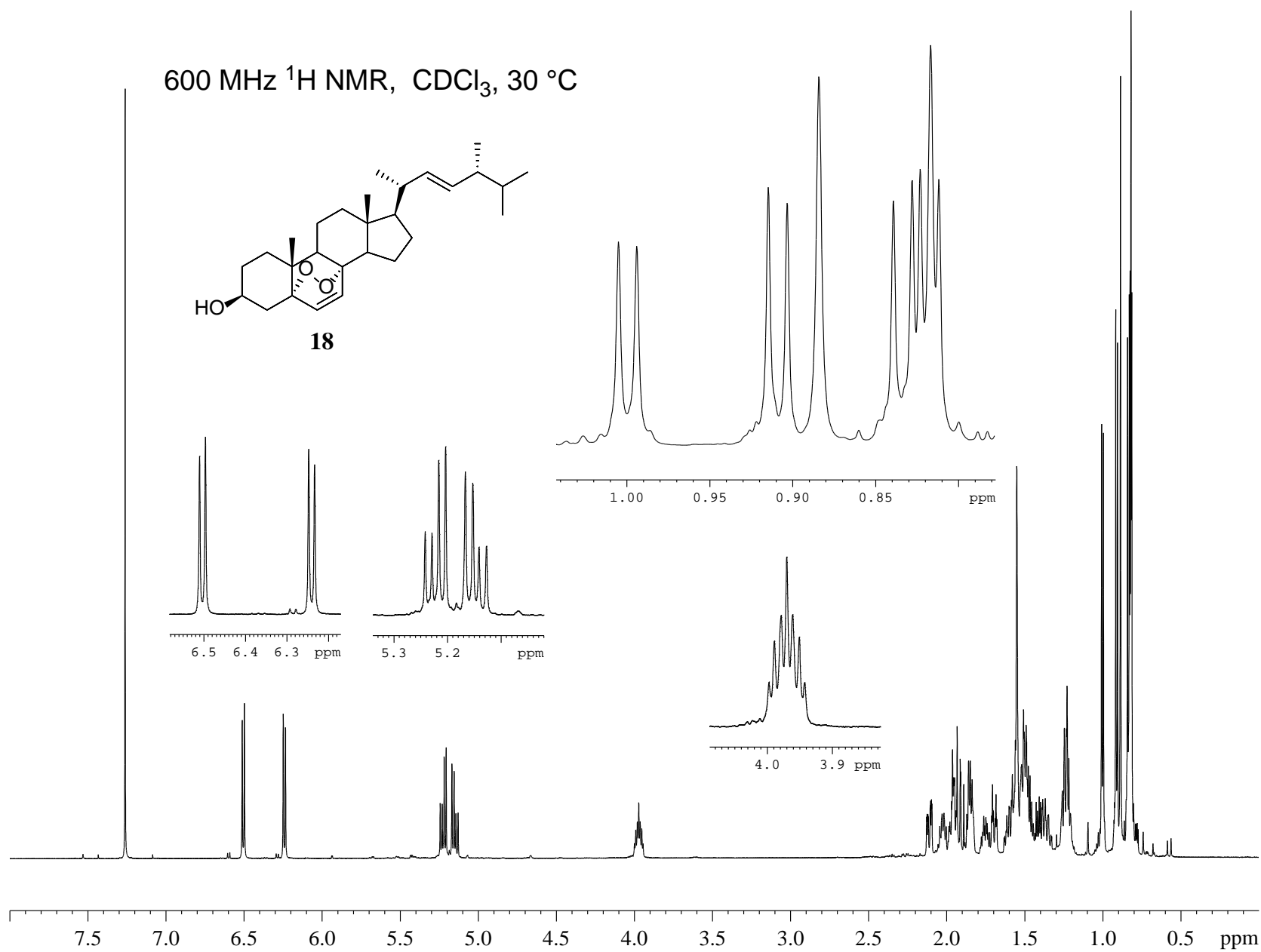
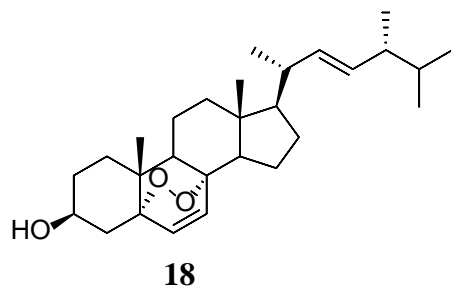
Future research should include the biological studies of all the synthesized sterols. Feeding experiments already confirmed the inhibition of phytosterol metabolism in invertebrates by the synthesized simple analogs of petrosterol (not yet published). More tropical orchids can be investigated as potential source of unusual sterols with a highly substituted side chain cyclopropane. Biological studies of those sterols should also help determine their functions in the orchids. Like in marine species, unusual sterols may also be used by plants as chemical defense against herbivorous insects. The visible-light activated thiyl radical oxidation of allylic alcohol needs to be investigated further to determine any roles of molecular oxygen in the formation of the thiyl radicals from phenyl disulfide. The reactions with low molecular weight substrates should be repeated on a larger scale with the standard solvent (9:1 hexanes/EtOAc) to determine if the reaction yields can be improved by changing the solvent.

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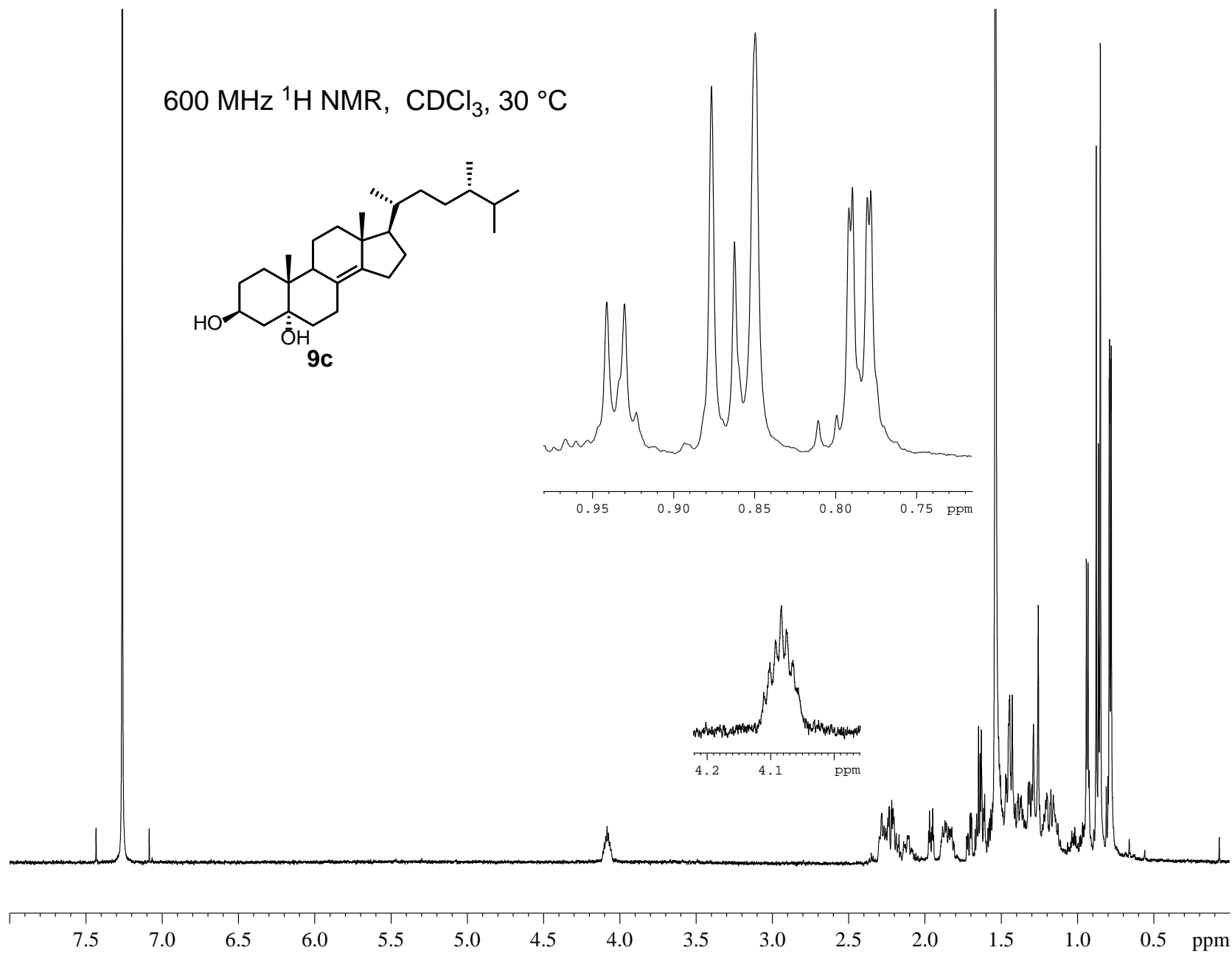
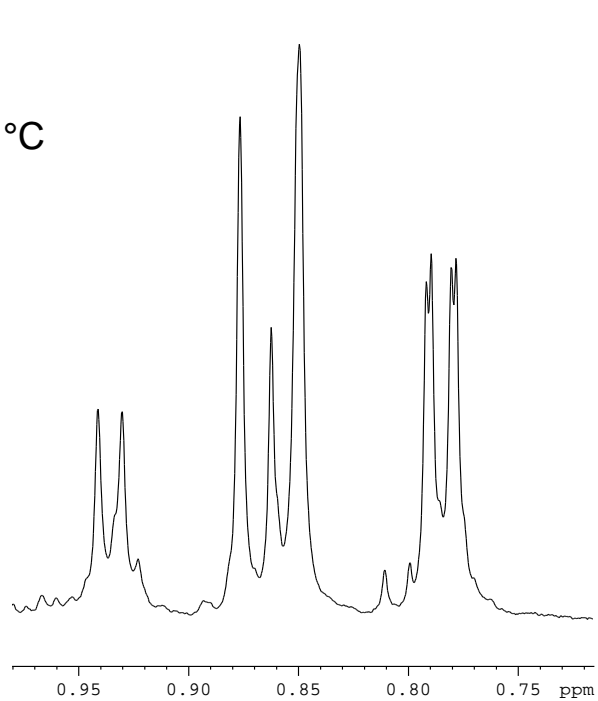
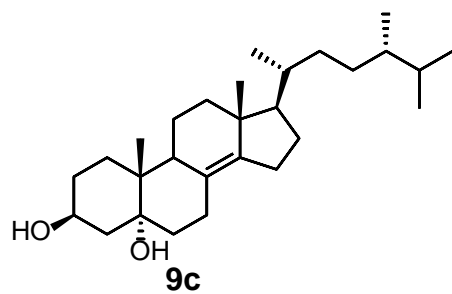
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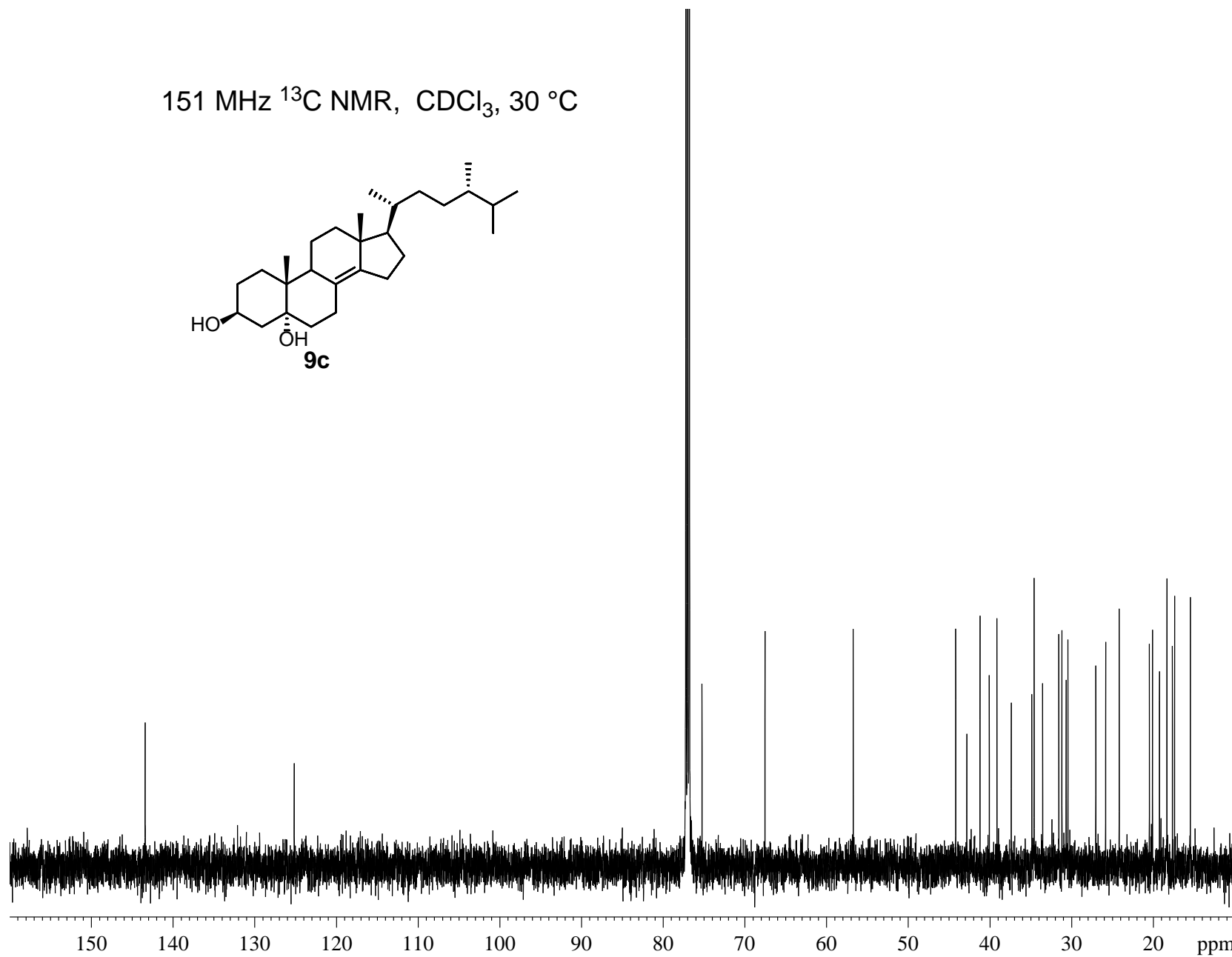
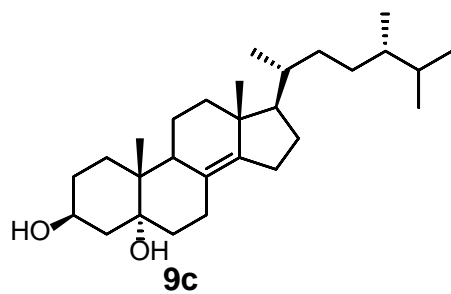
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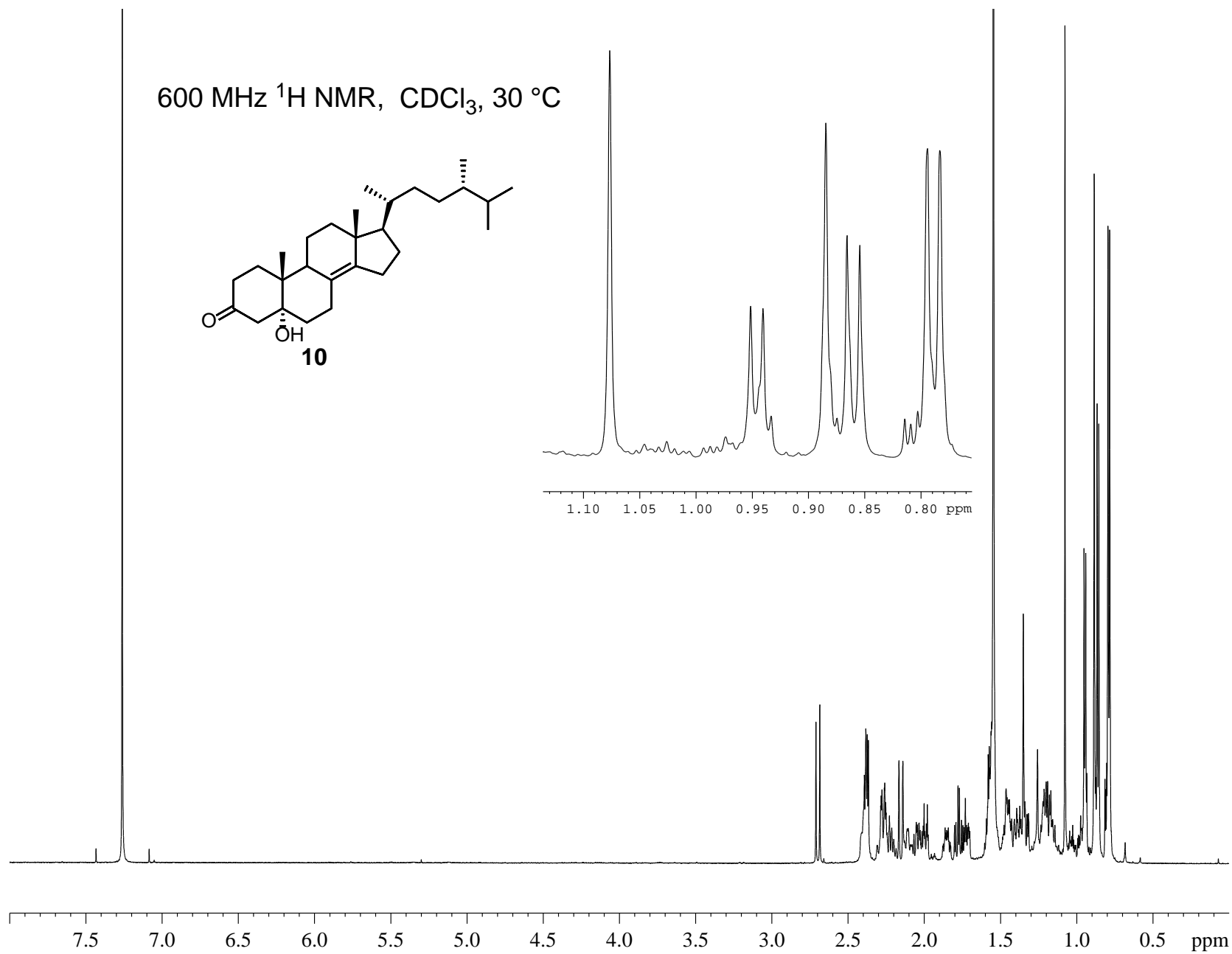
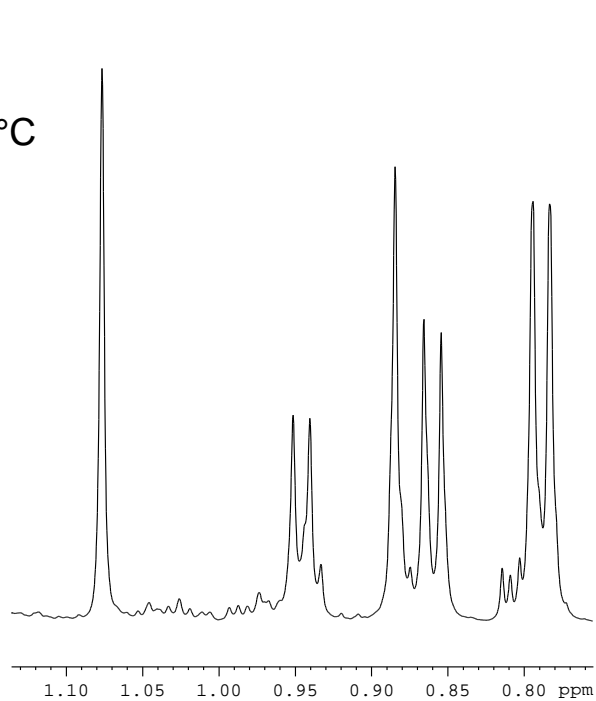
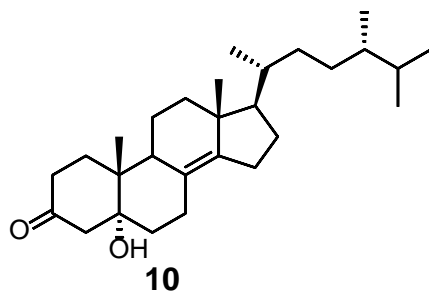
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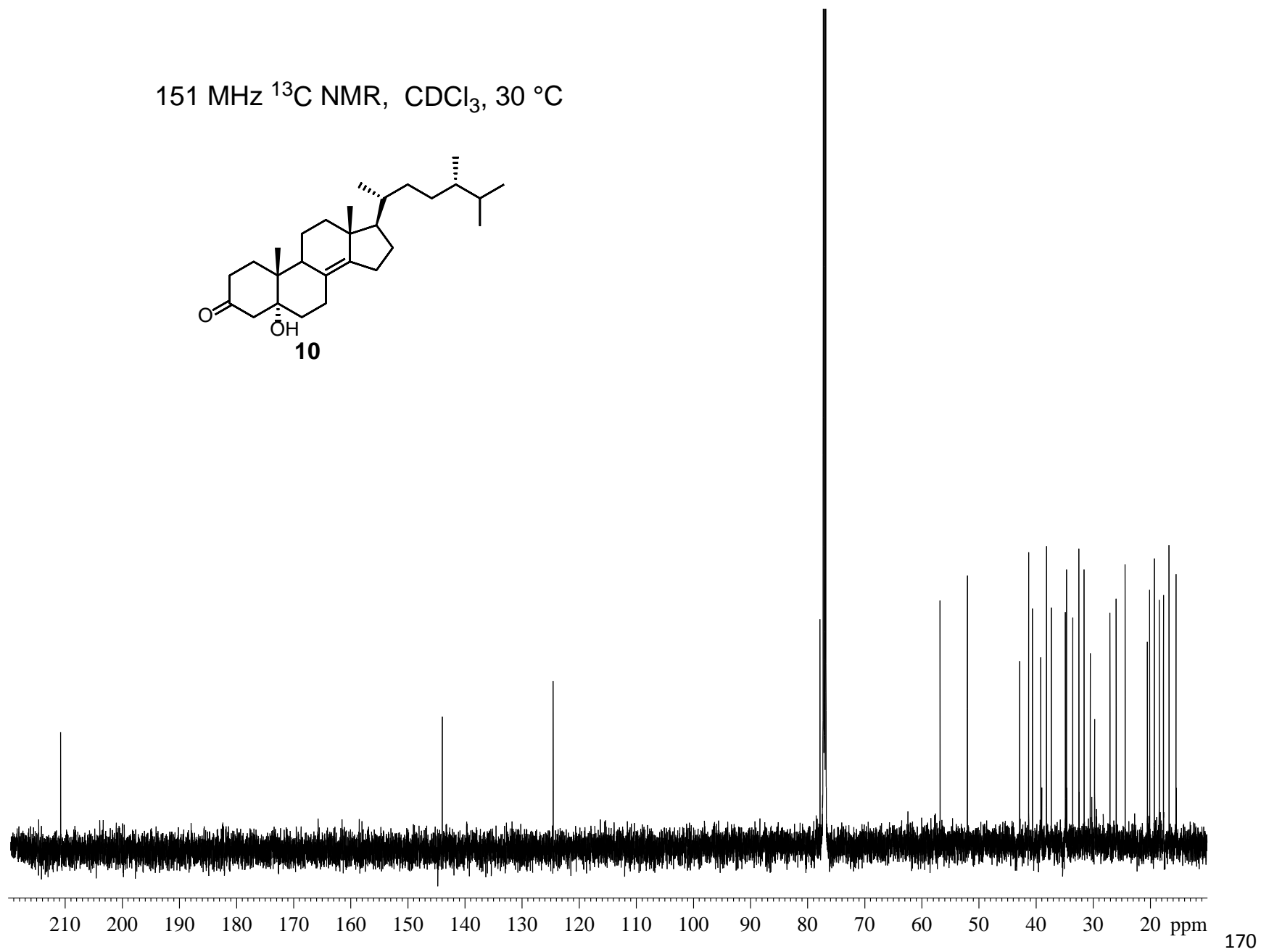
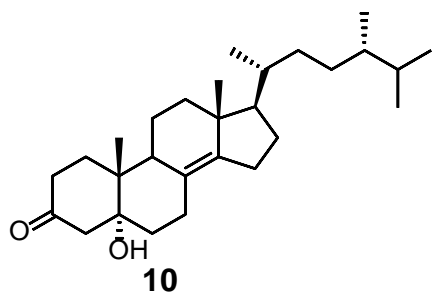
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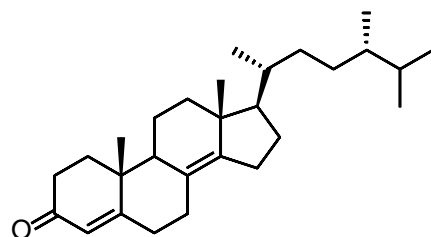
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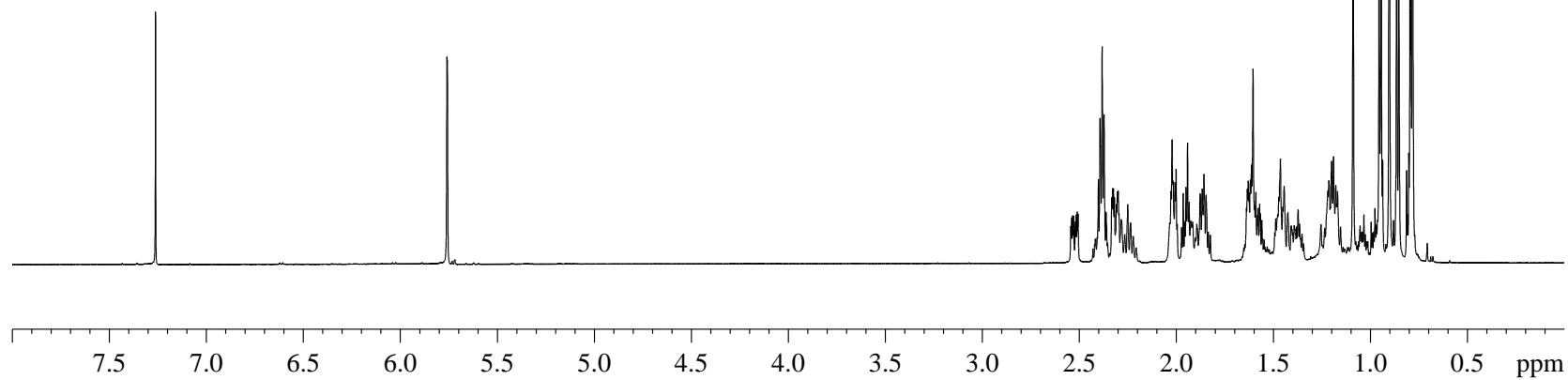
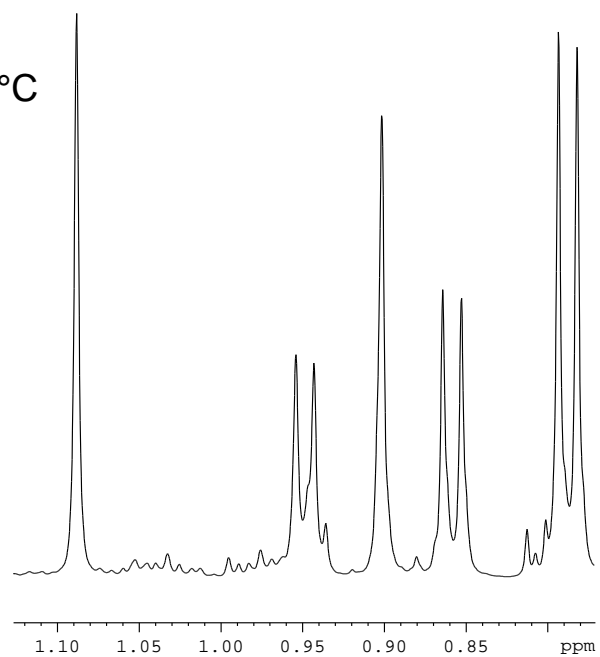
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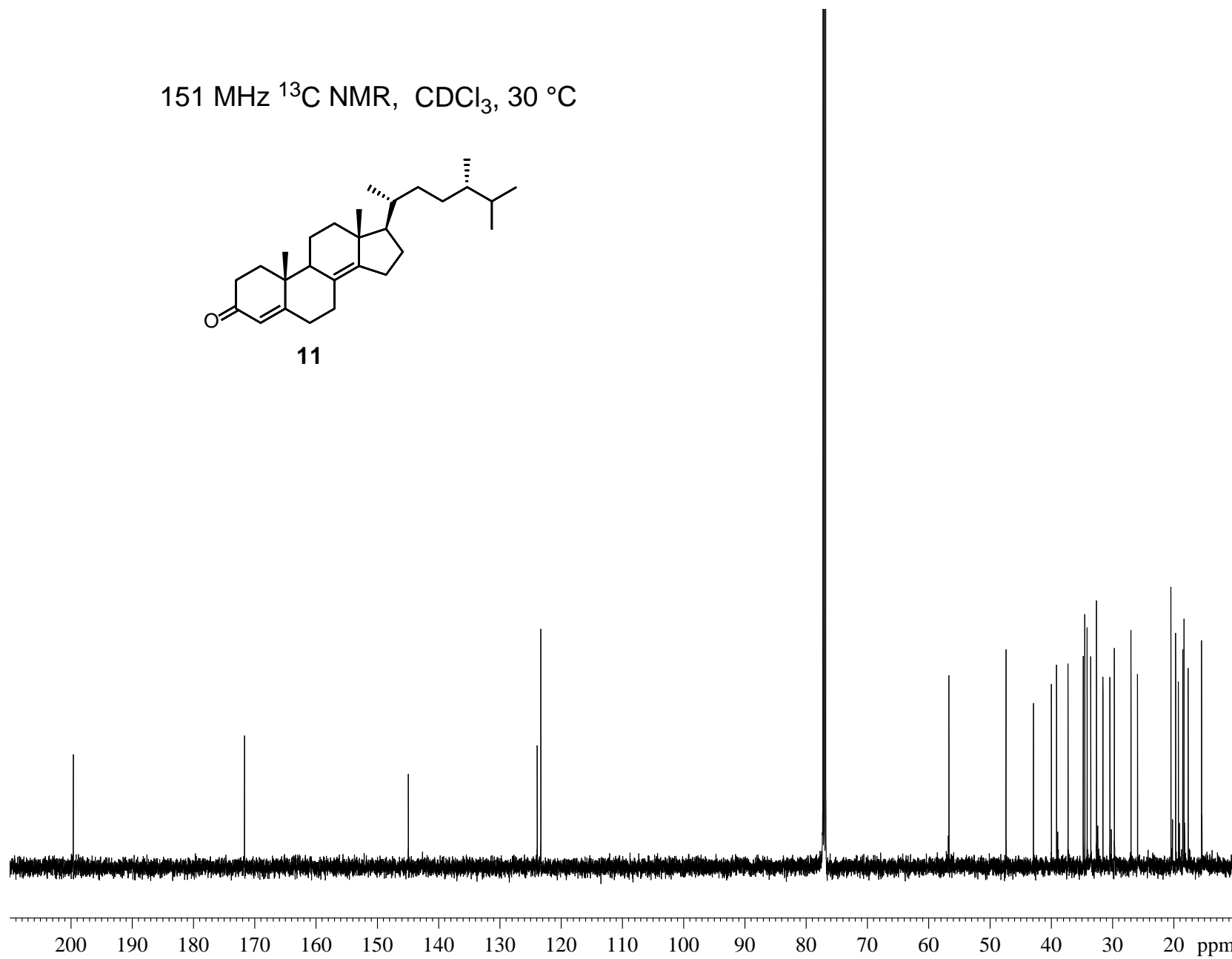
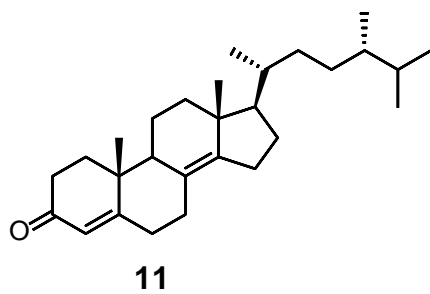
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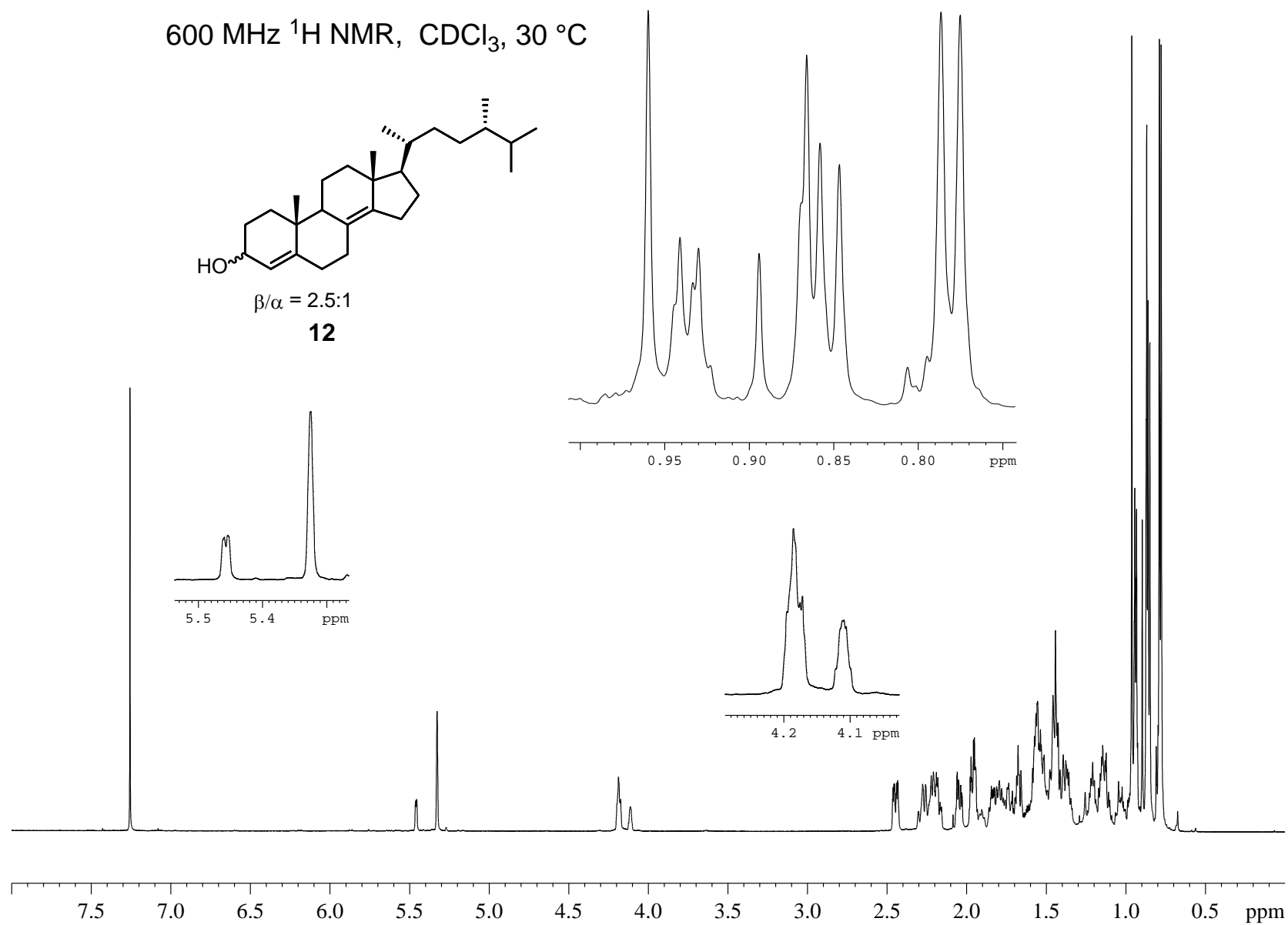
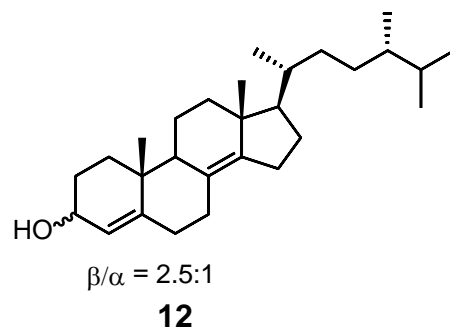
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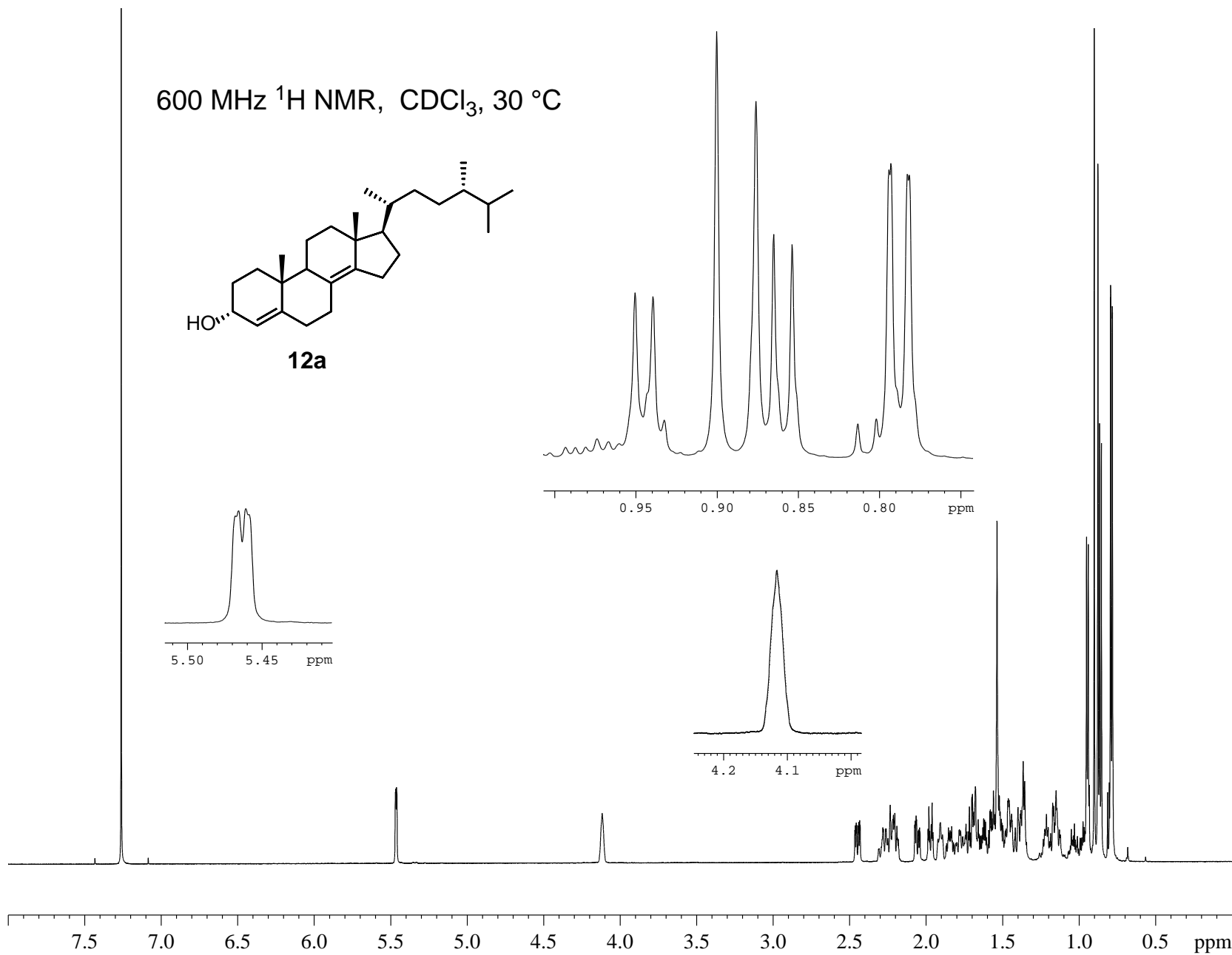
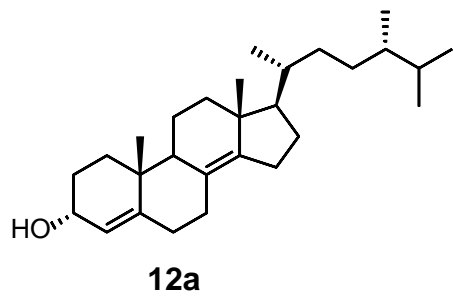


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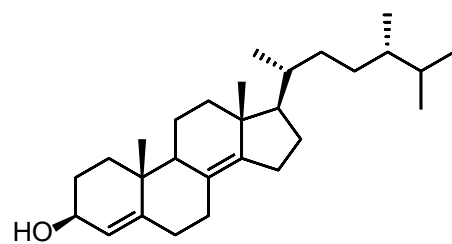


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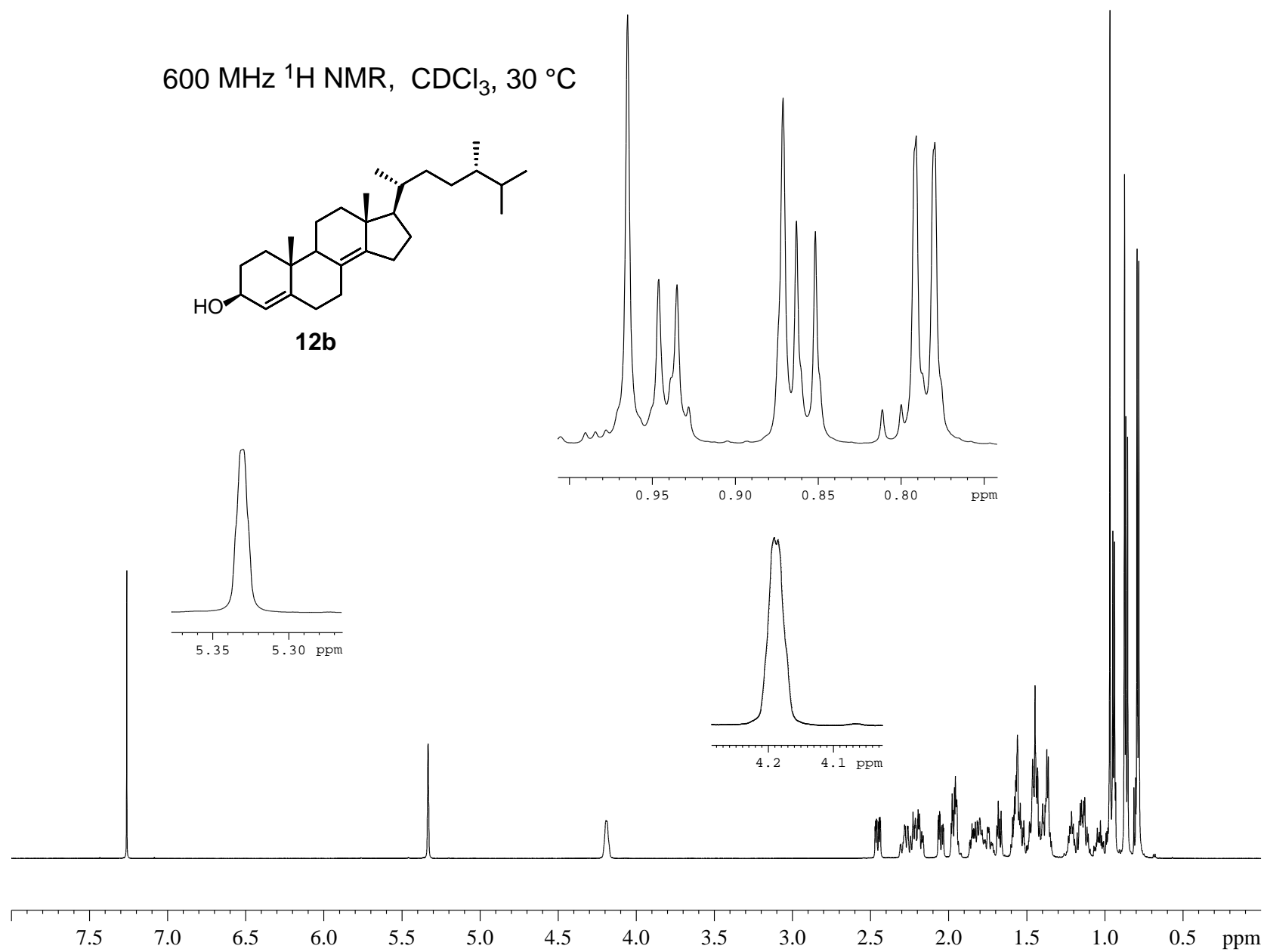


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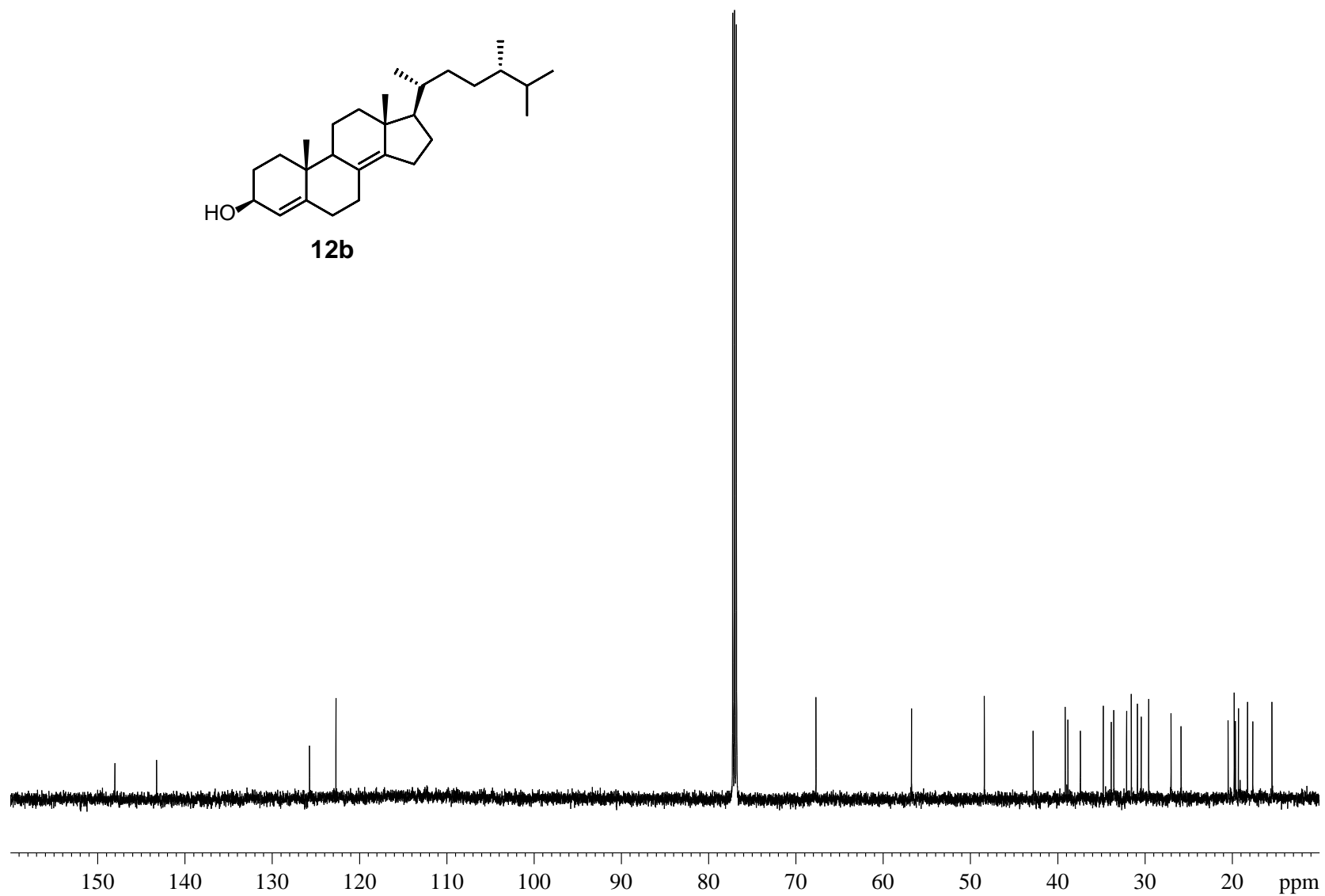
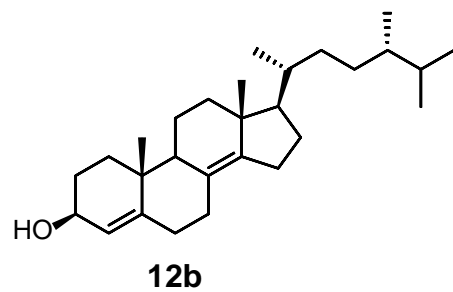
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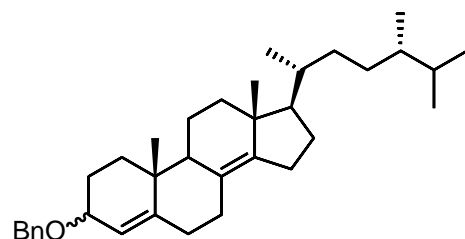
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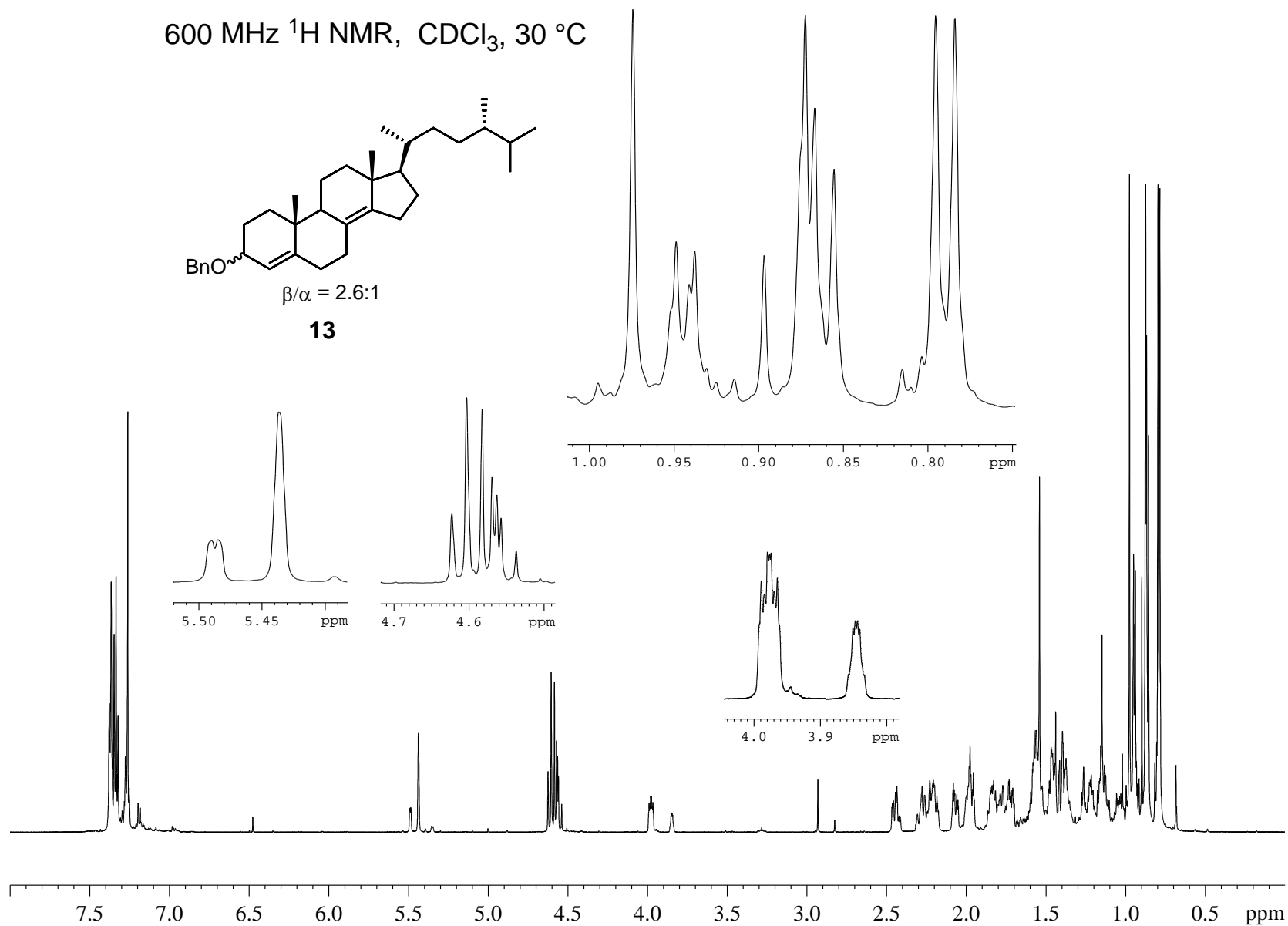


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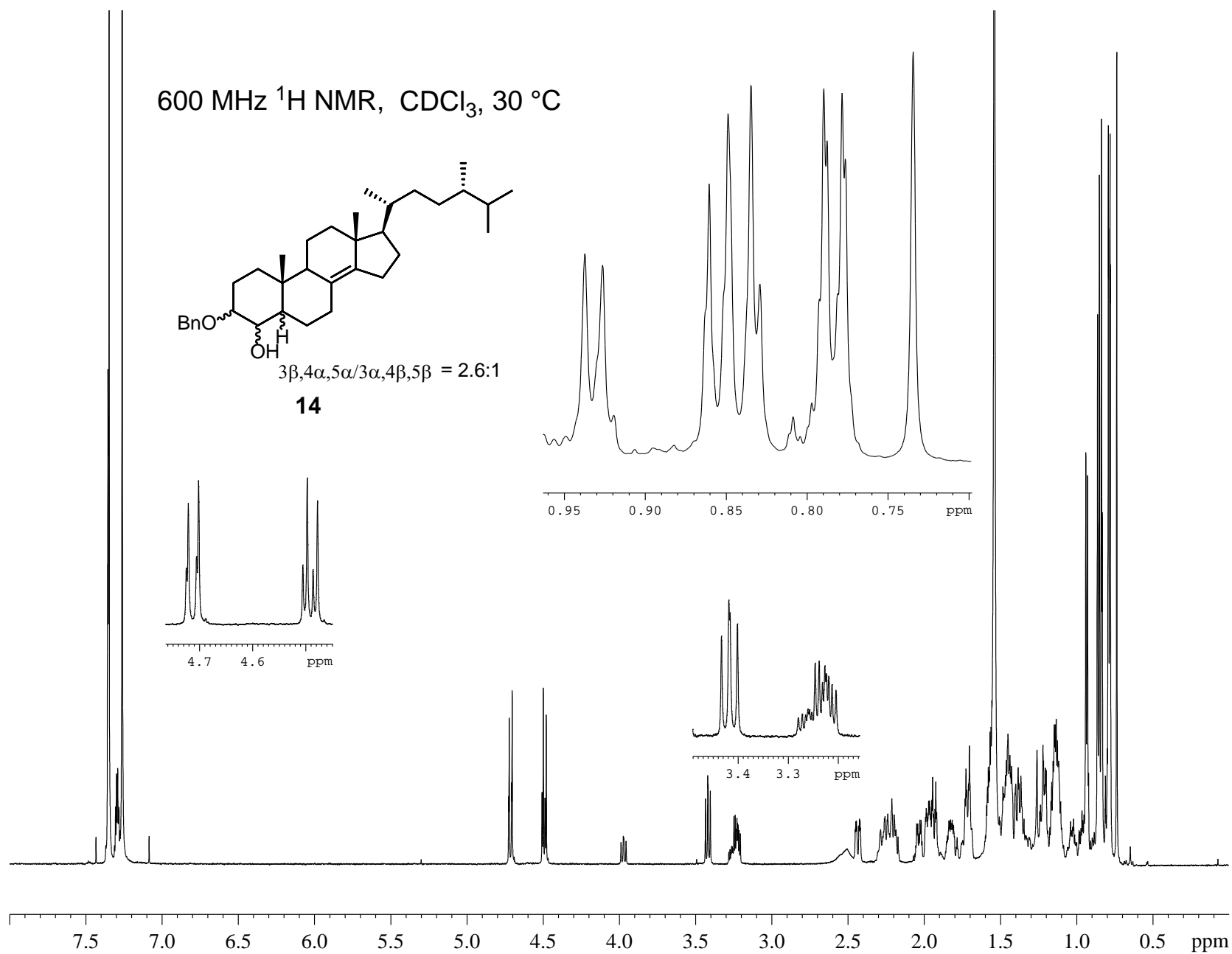
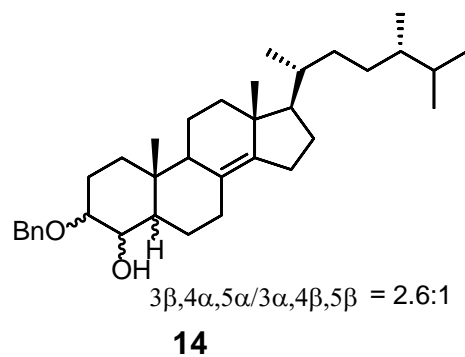


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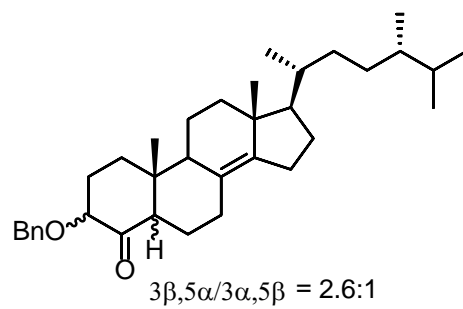
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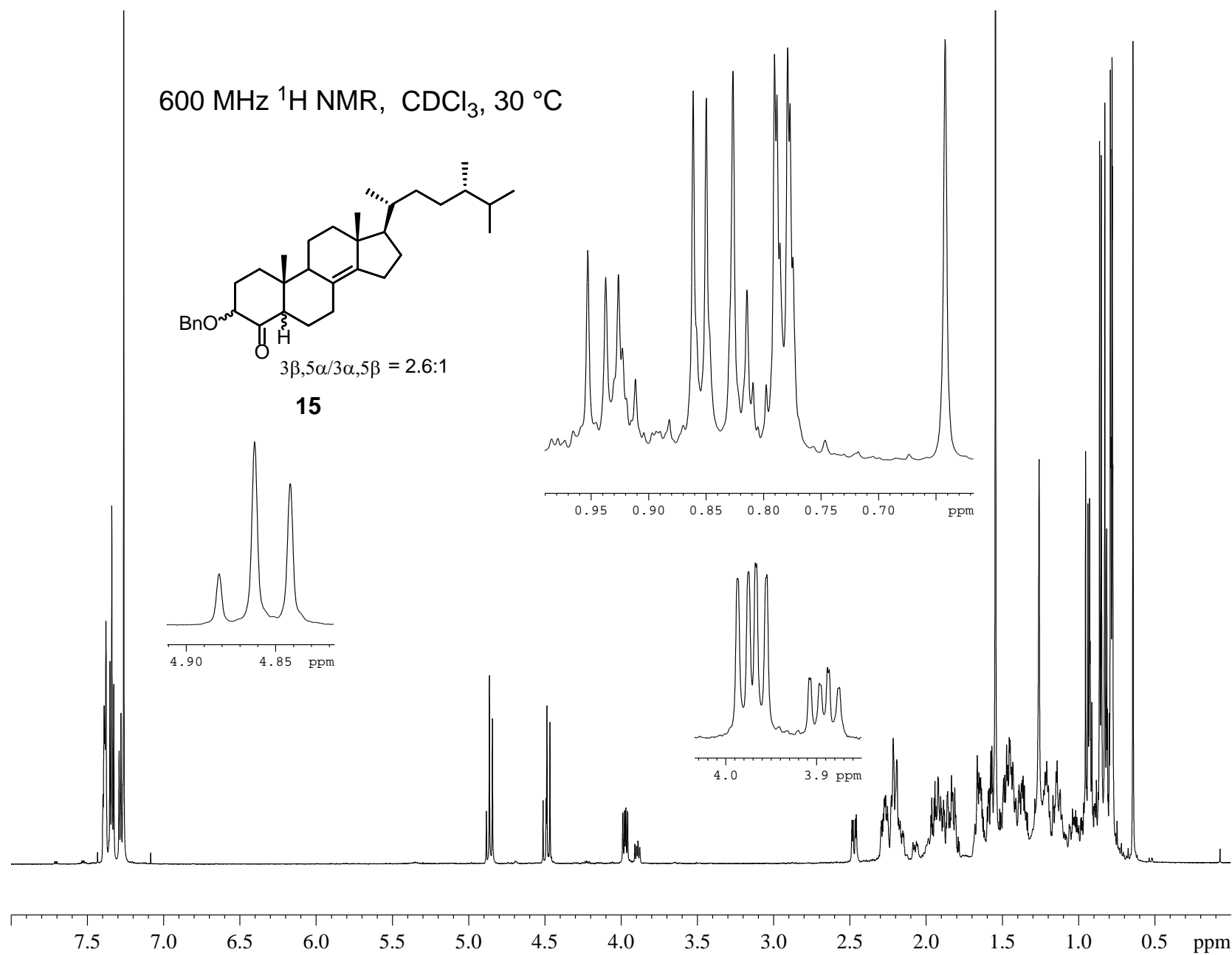
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15



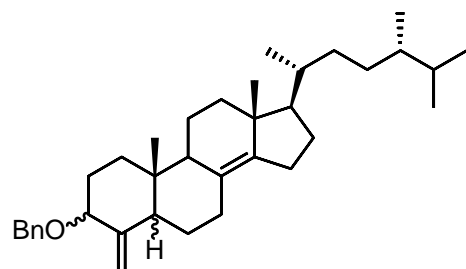
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15b

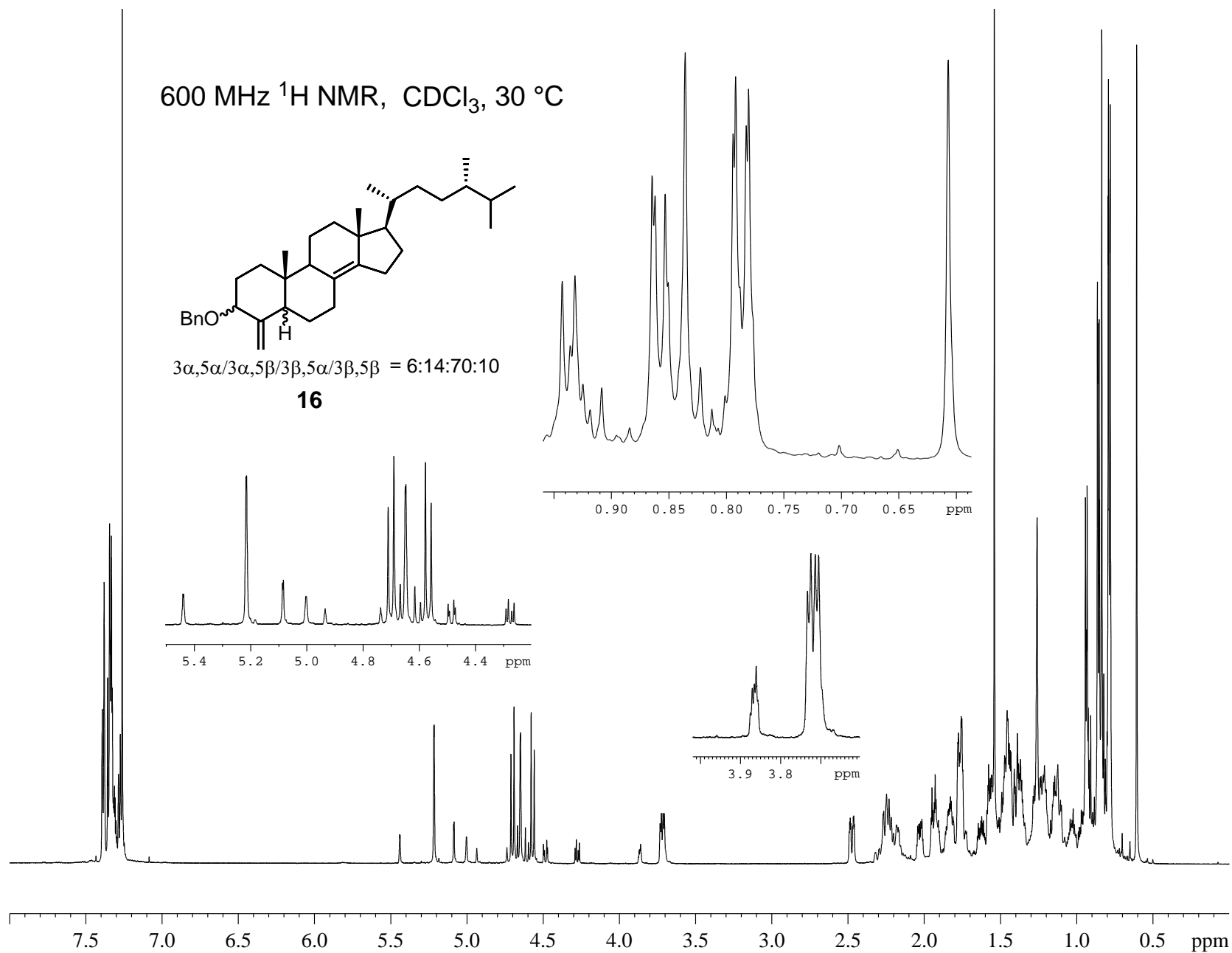


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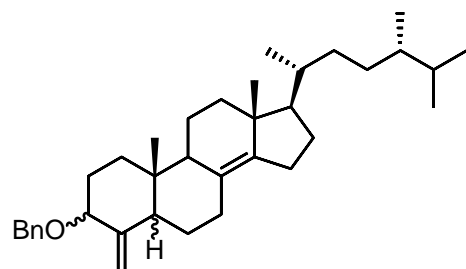


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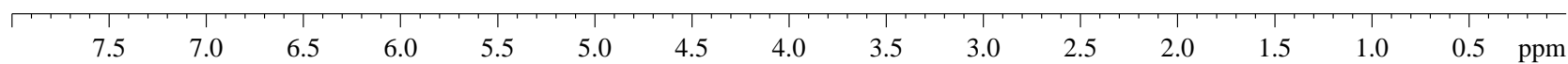
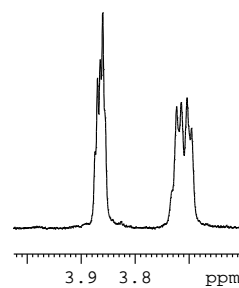
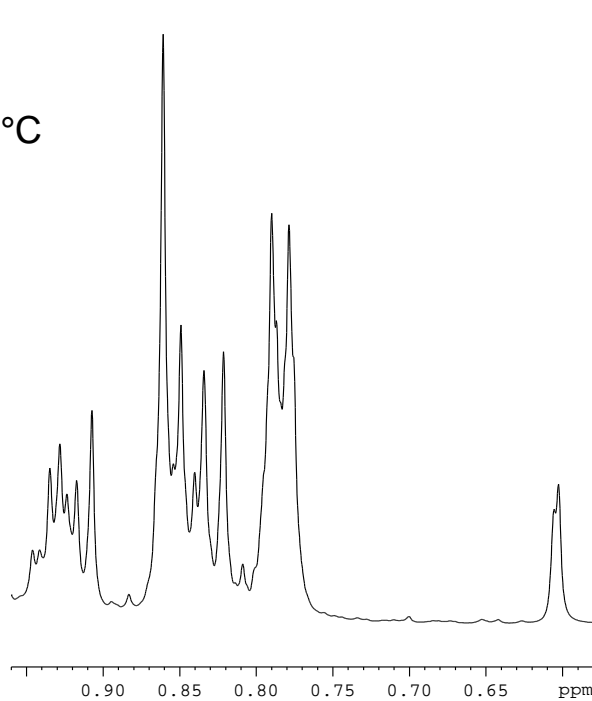
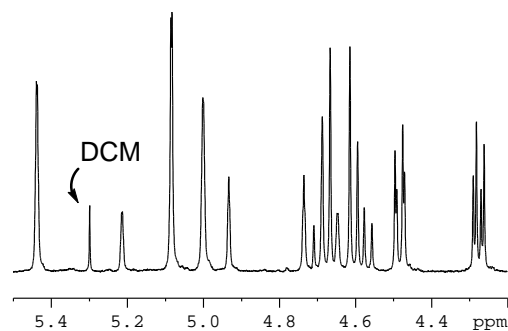


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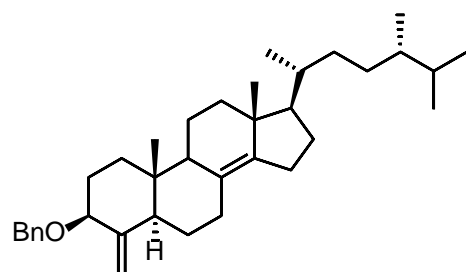


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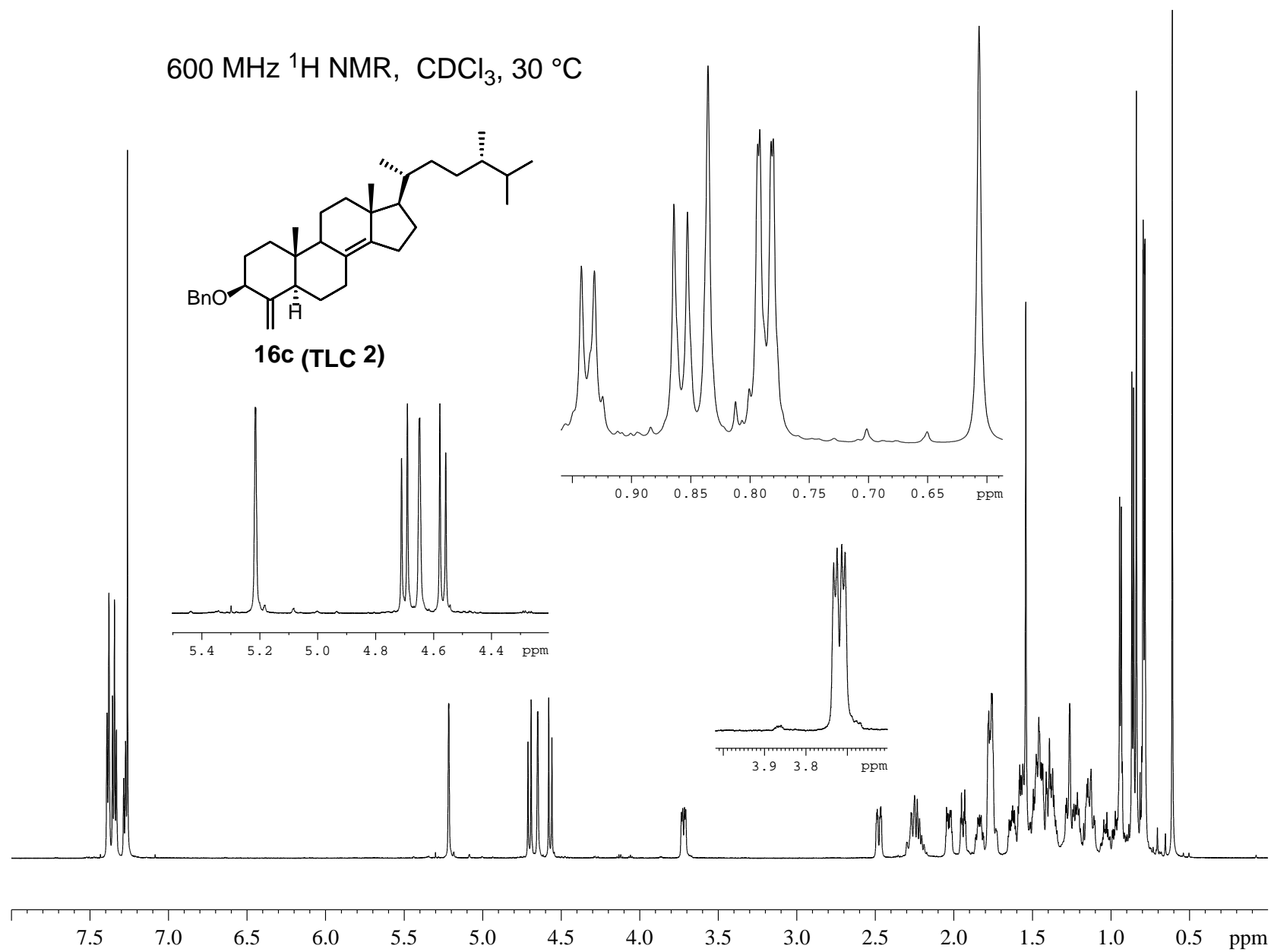
16 TLC 1



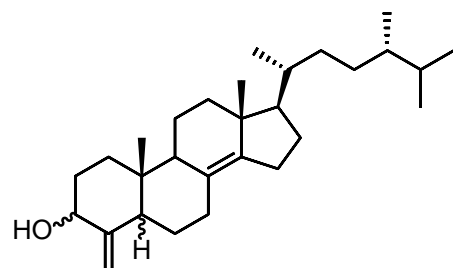
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16c (TLC 2)

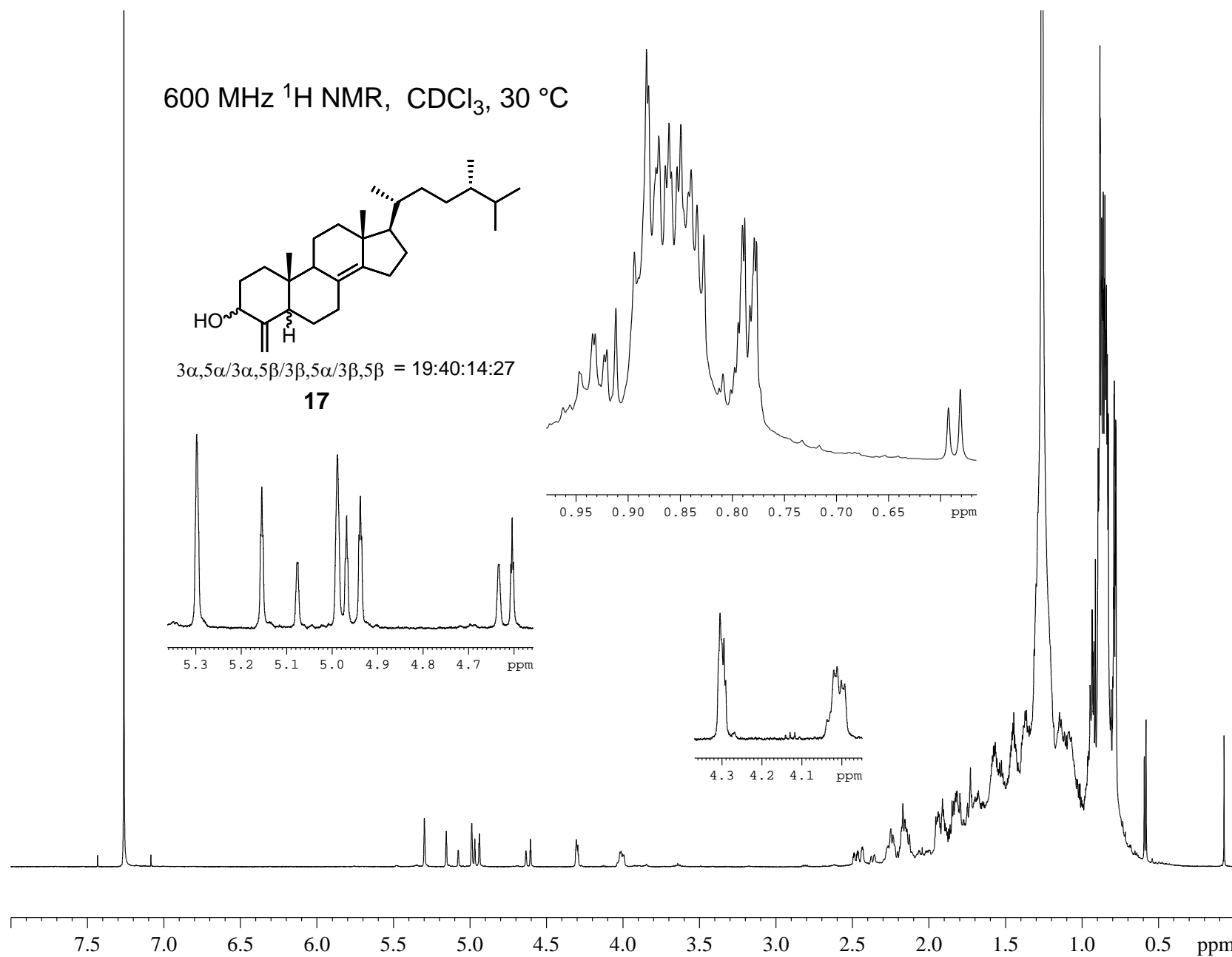


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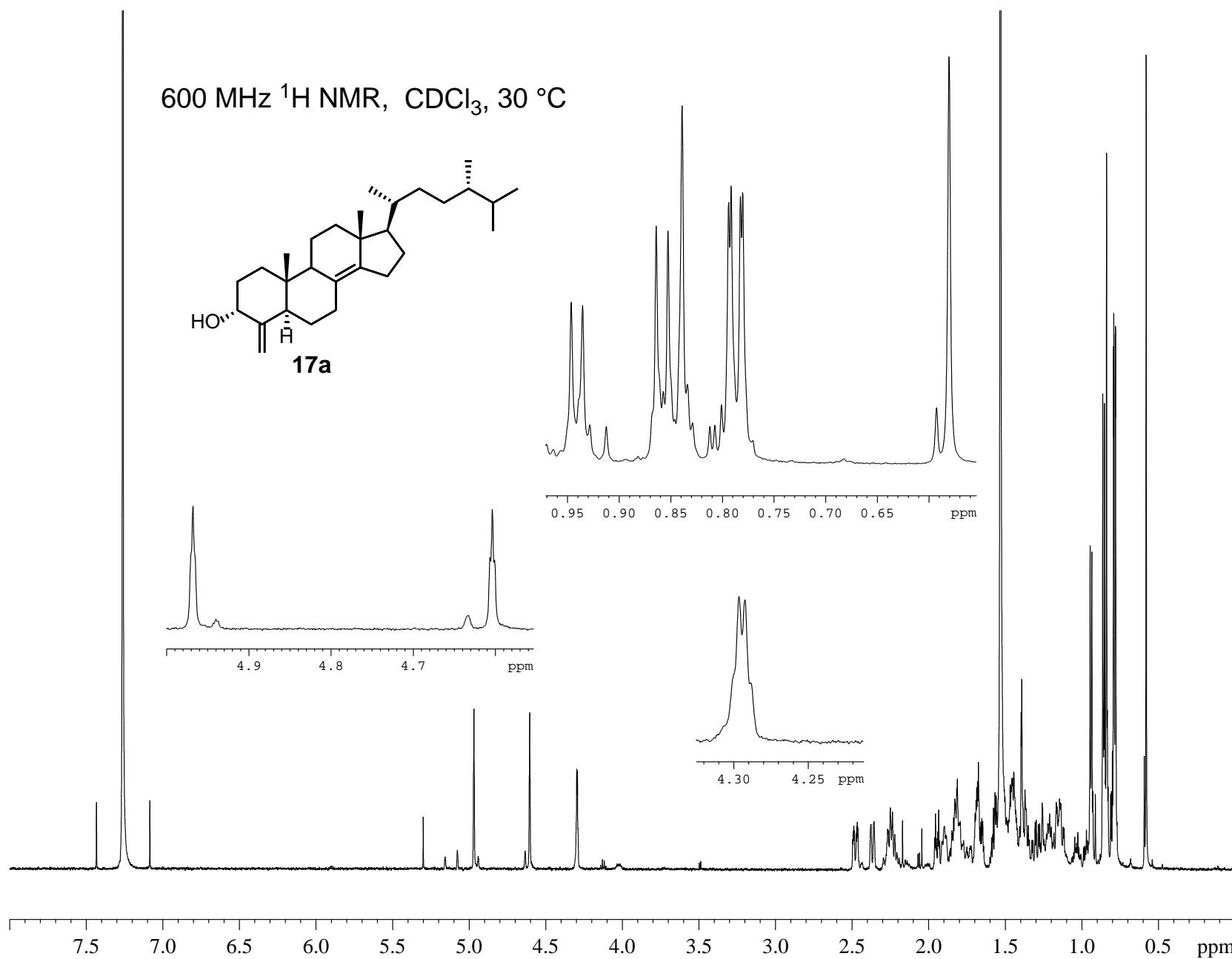
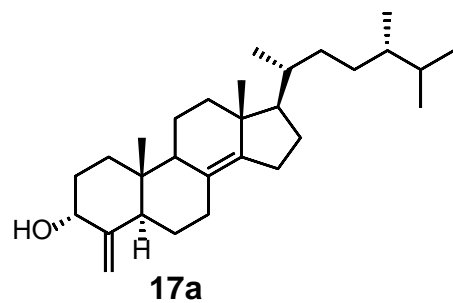


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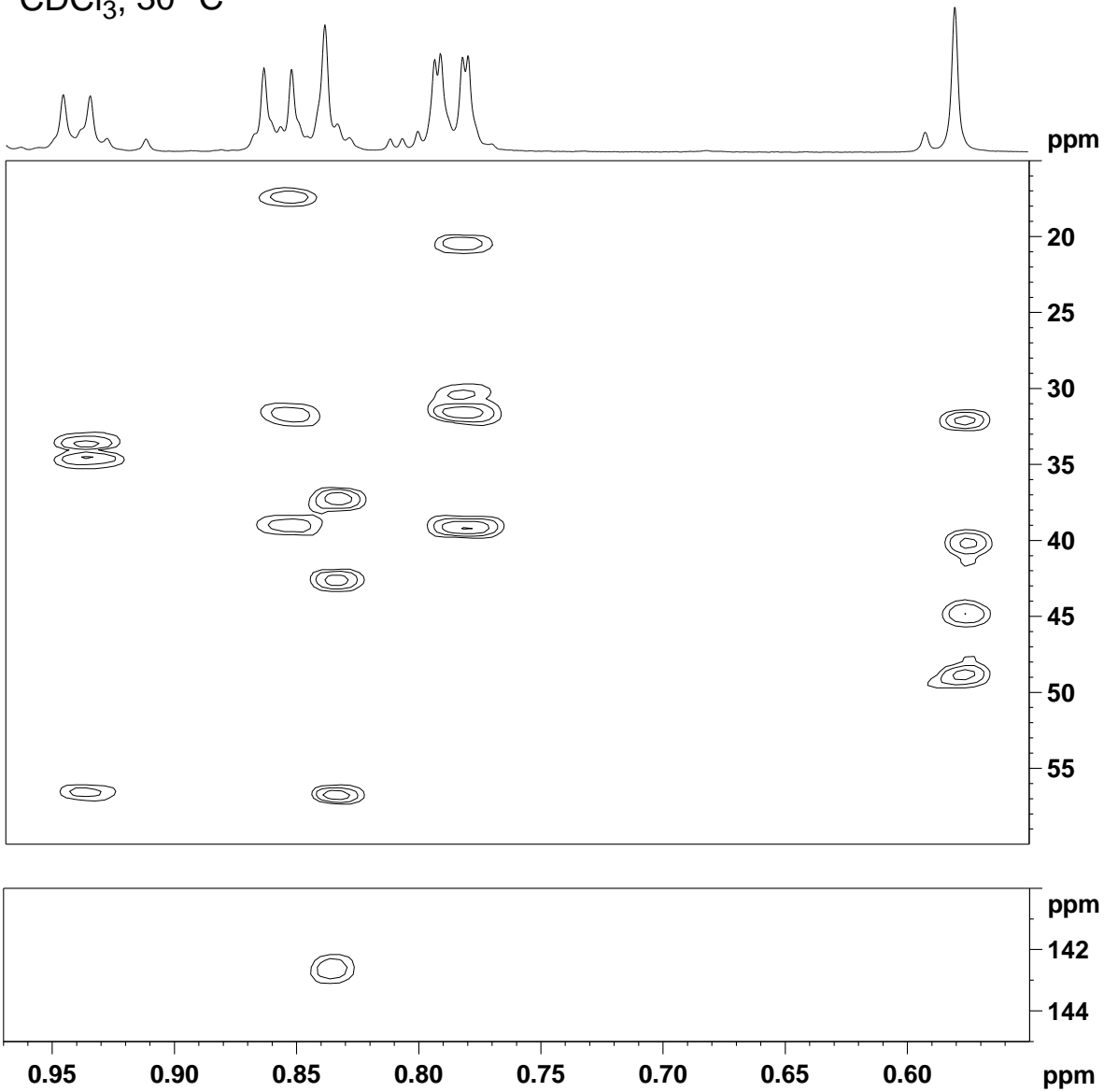
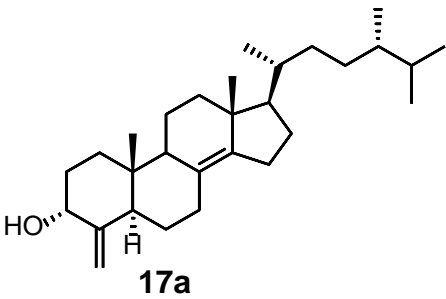
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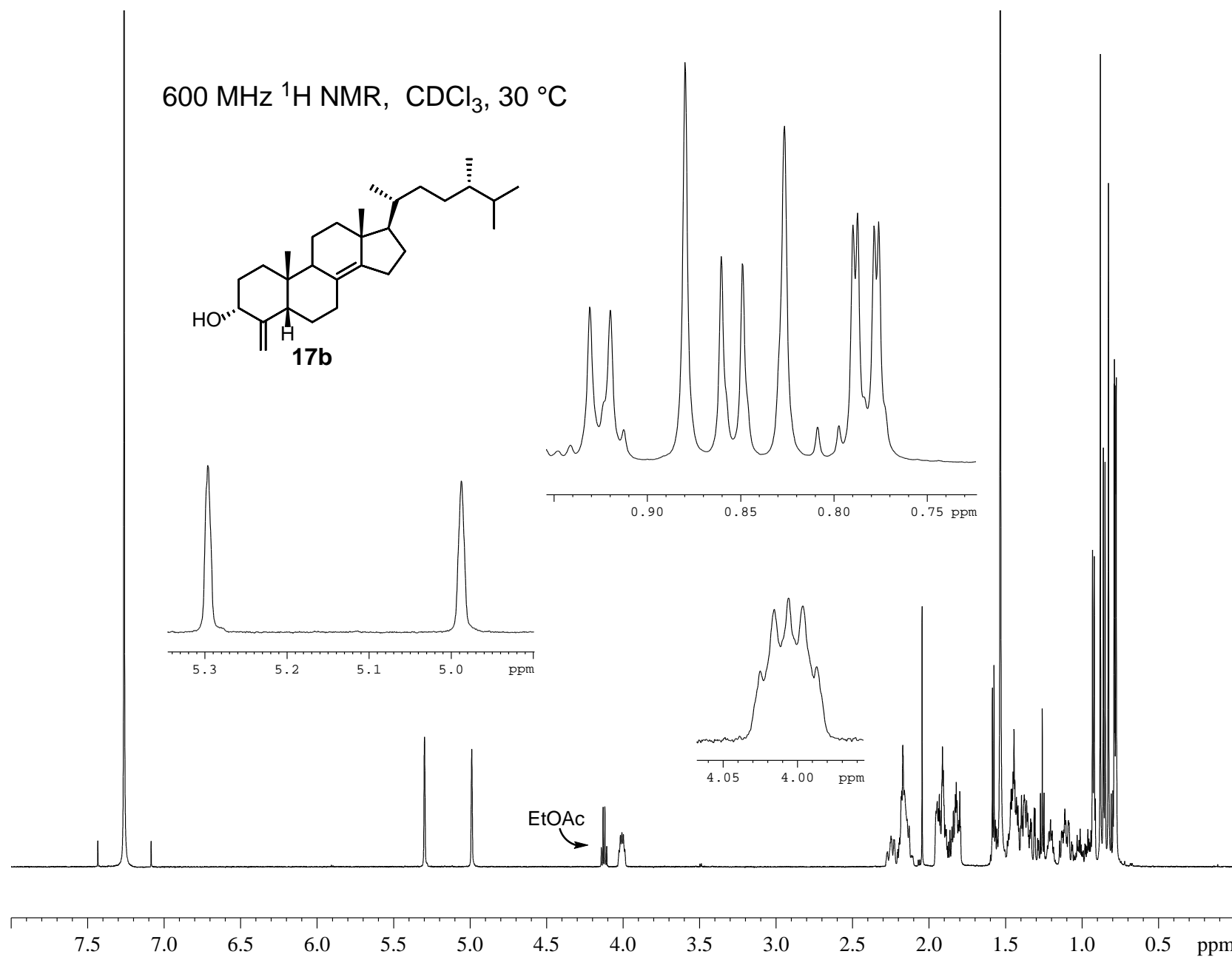
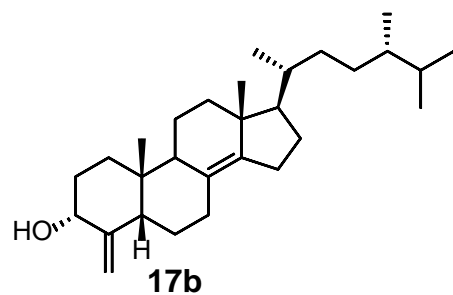
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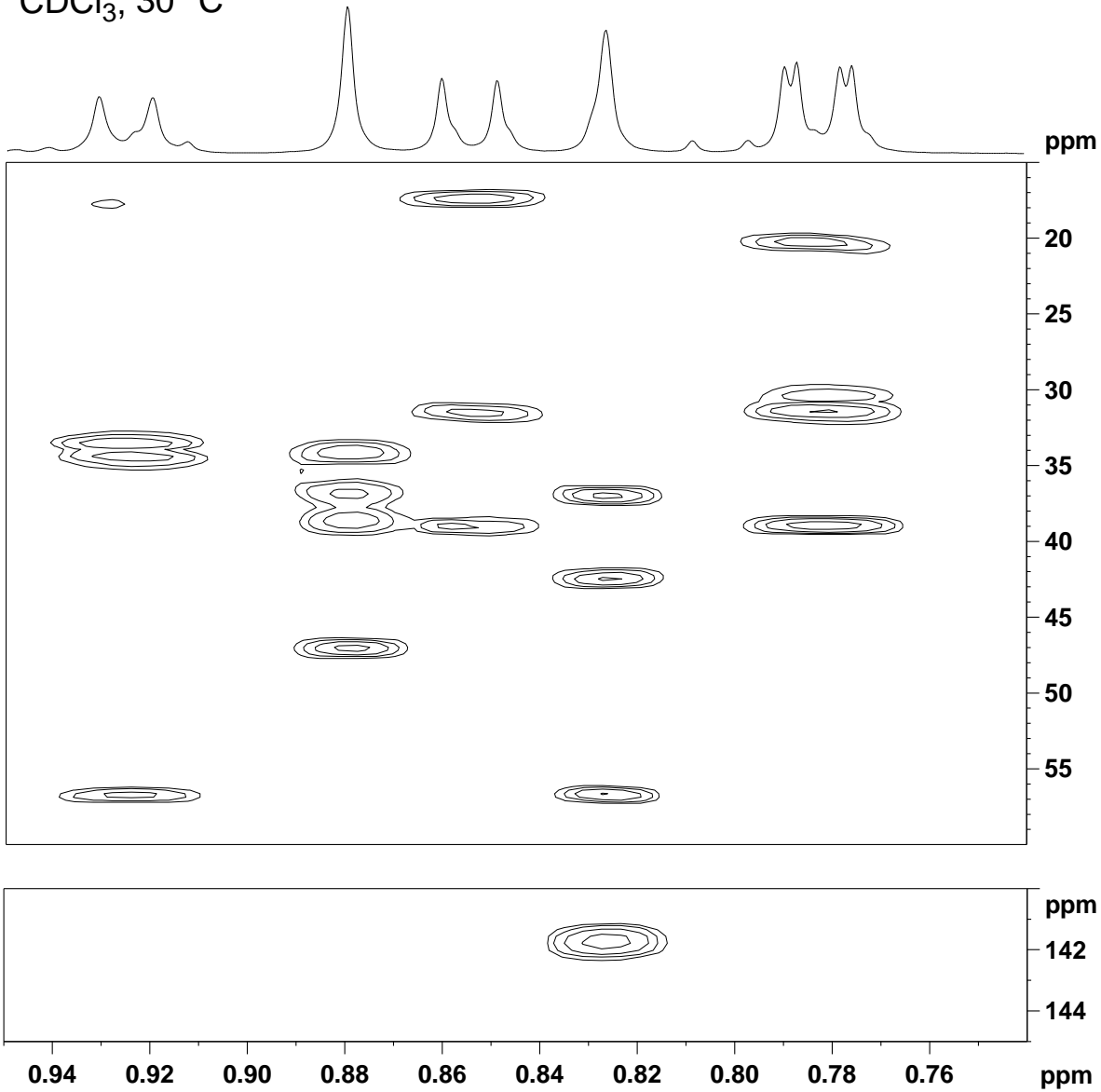
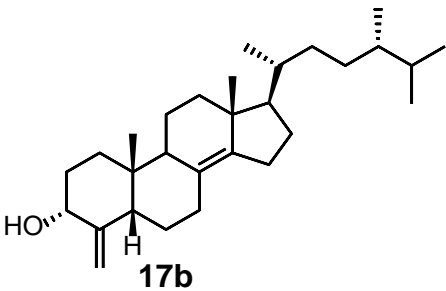
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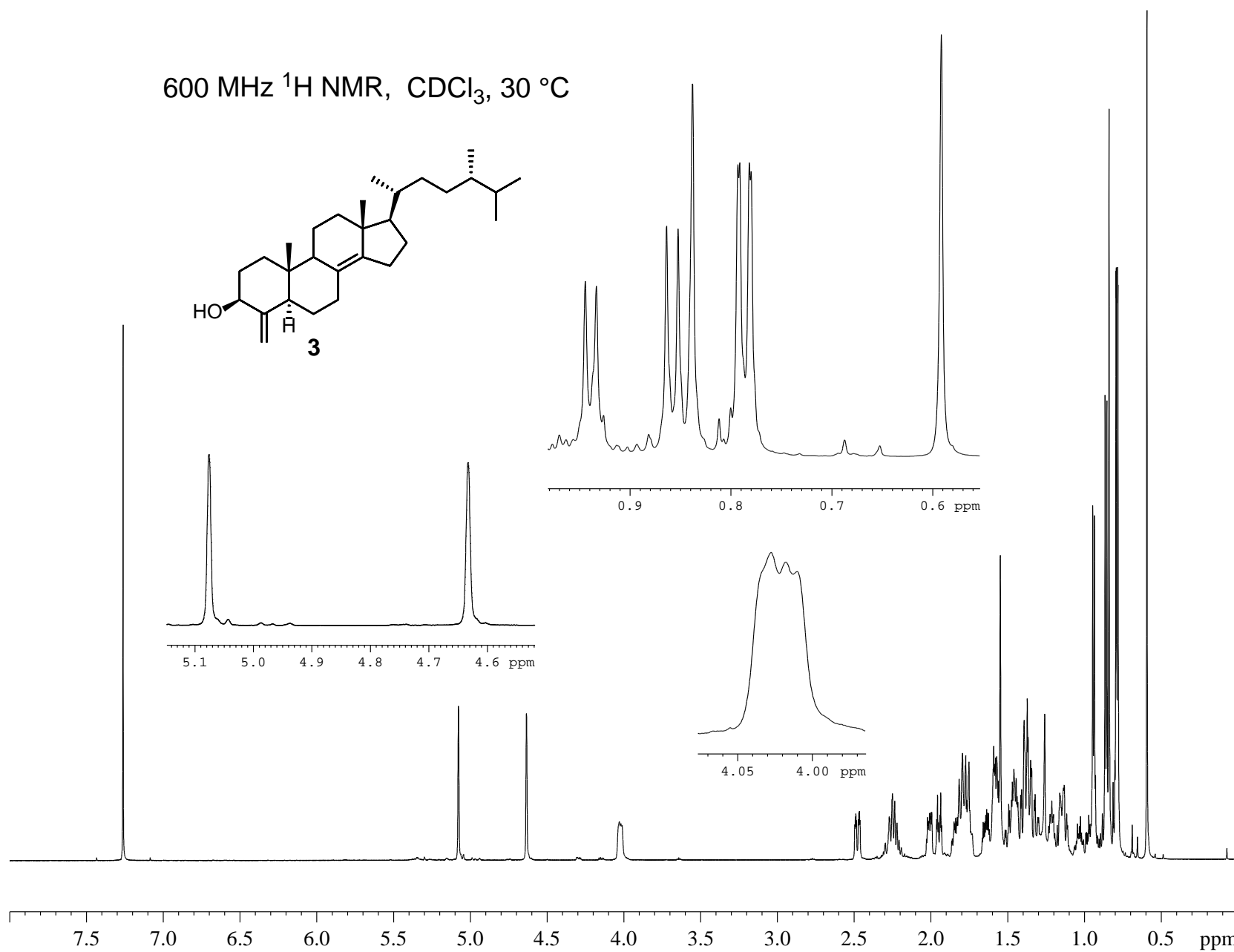
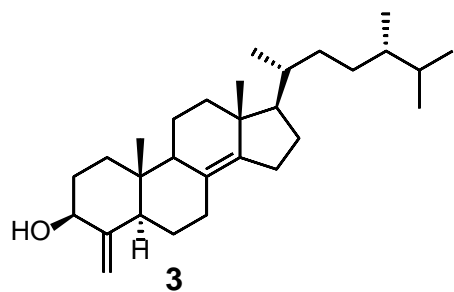
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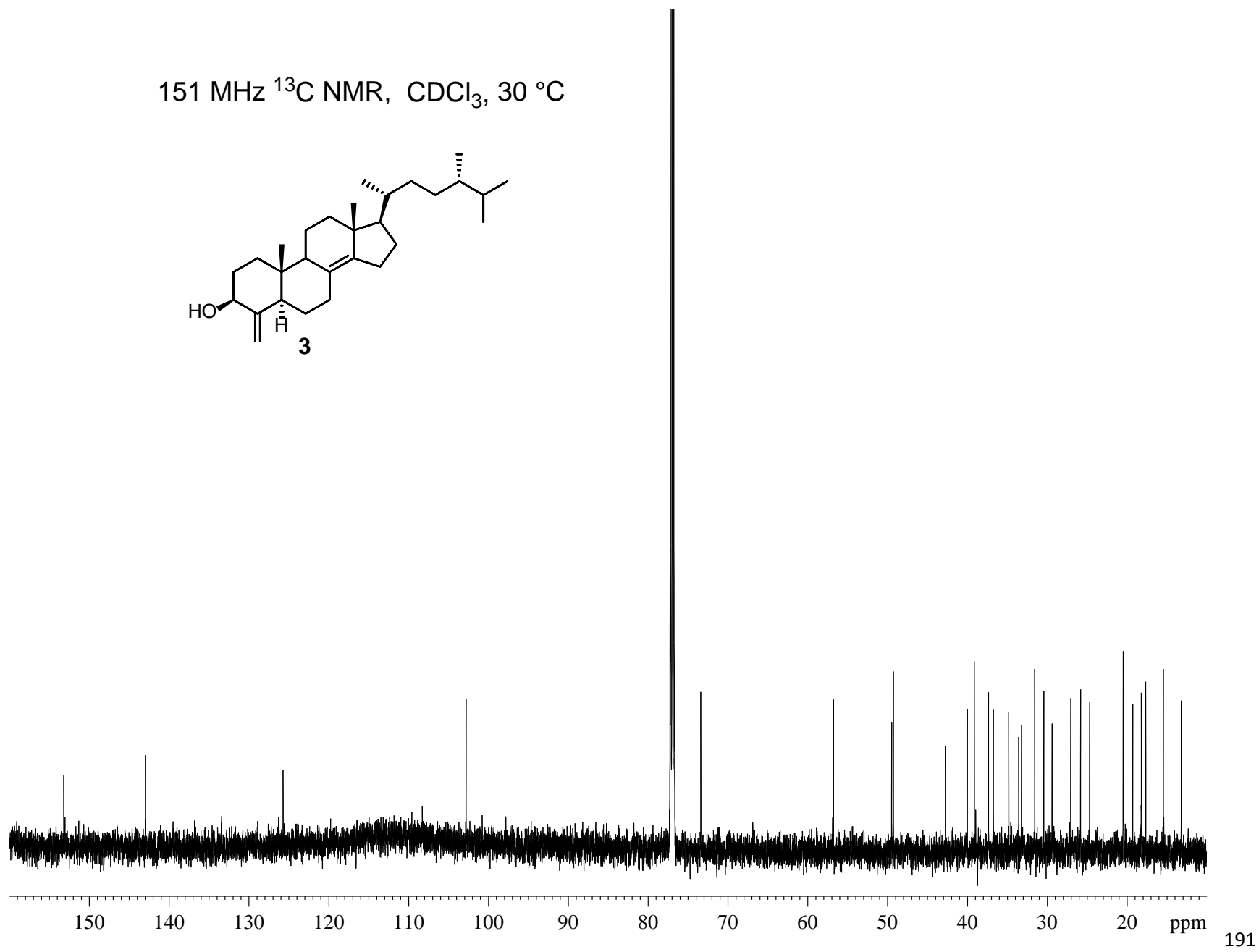
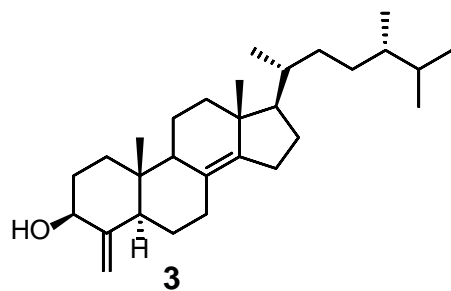
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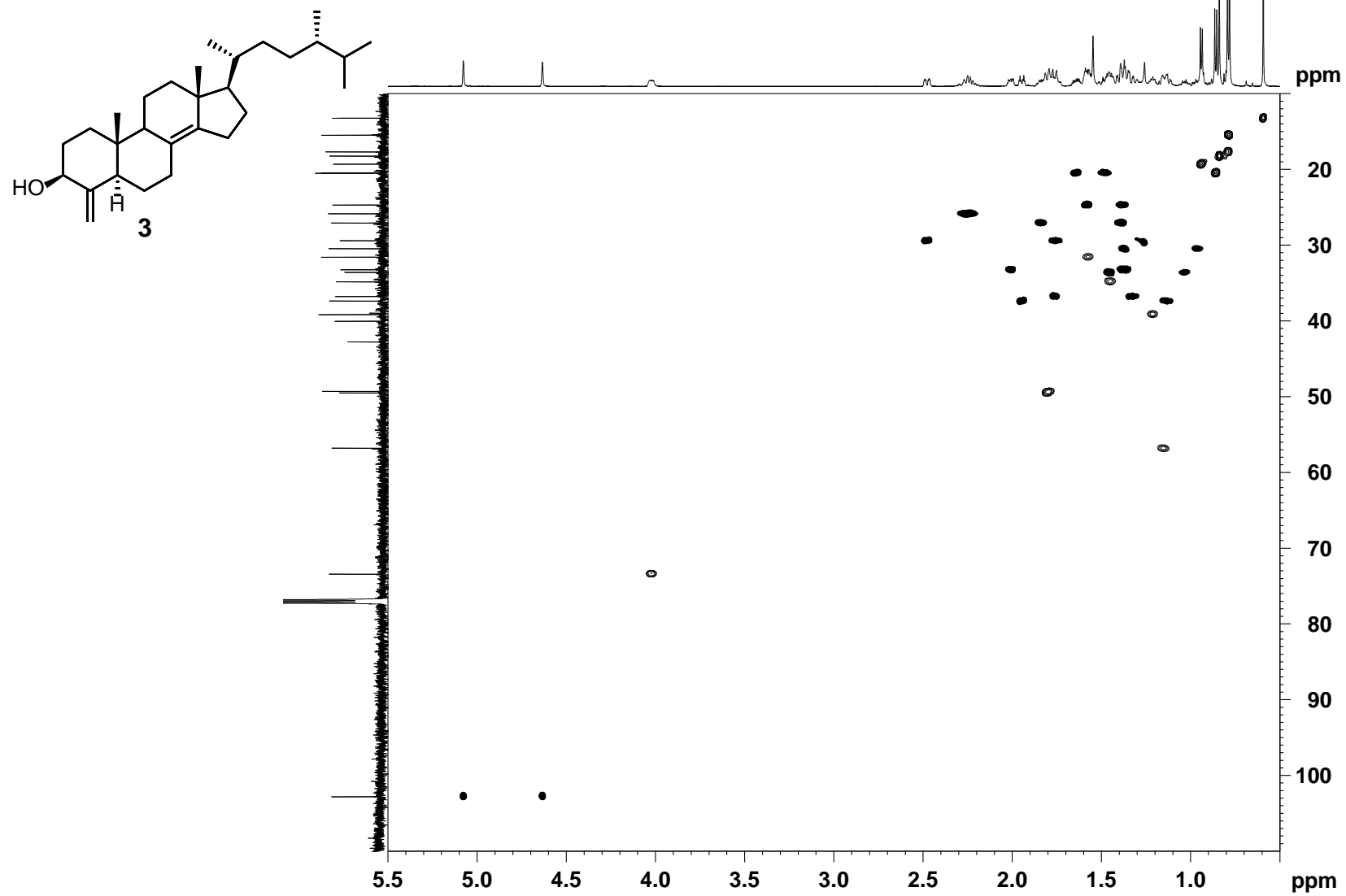
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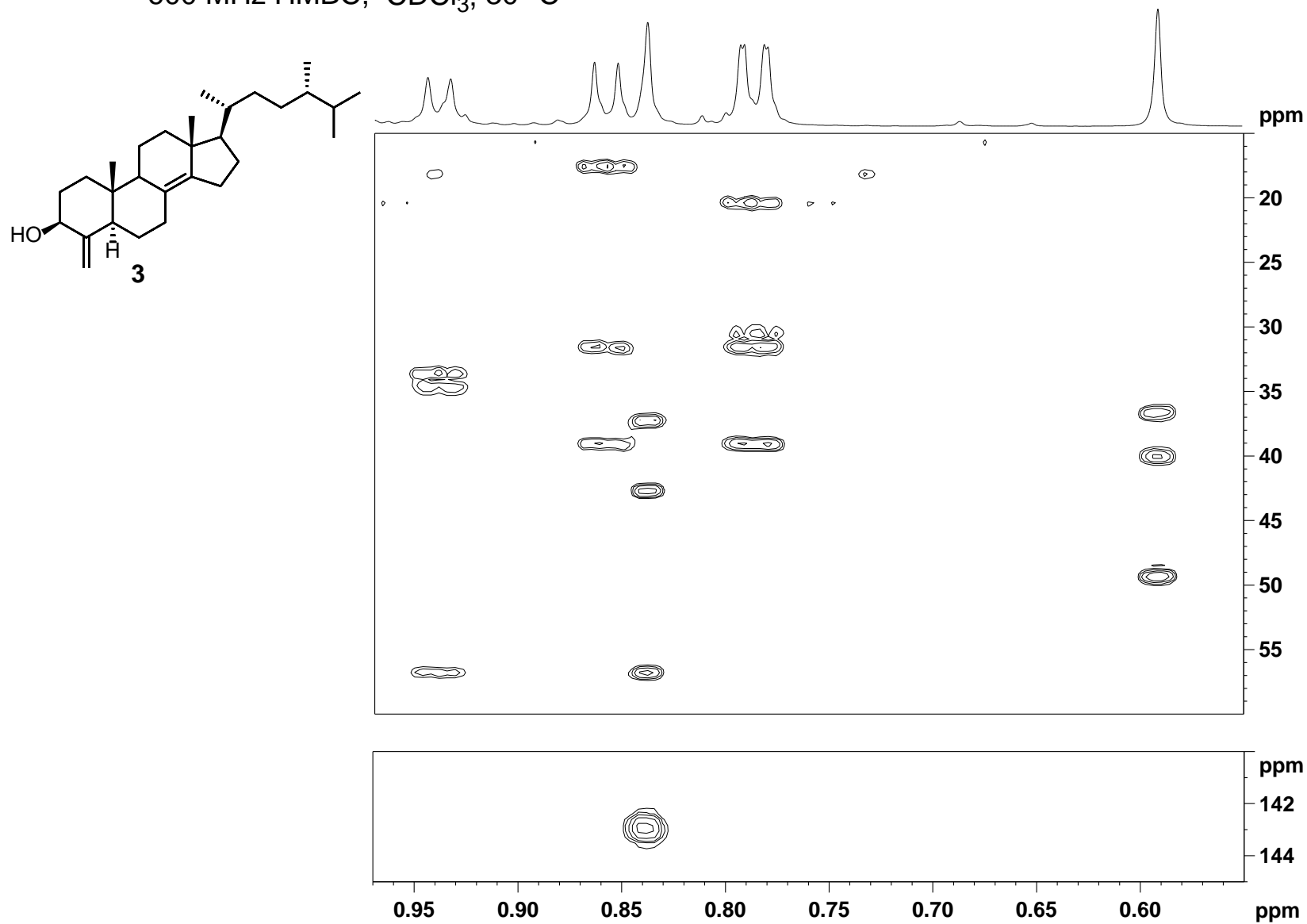
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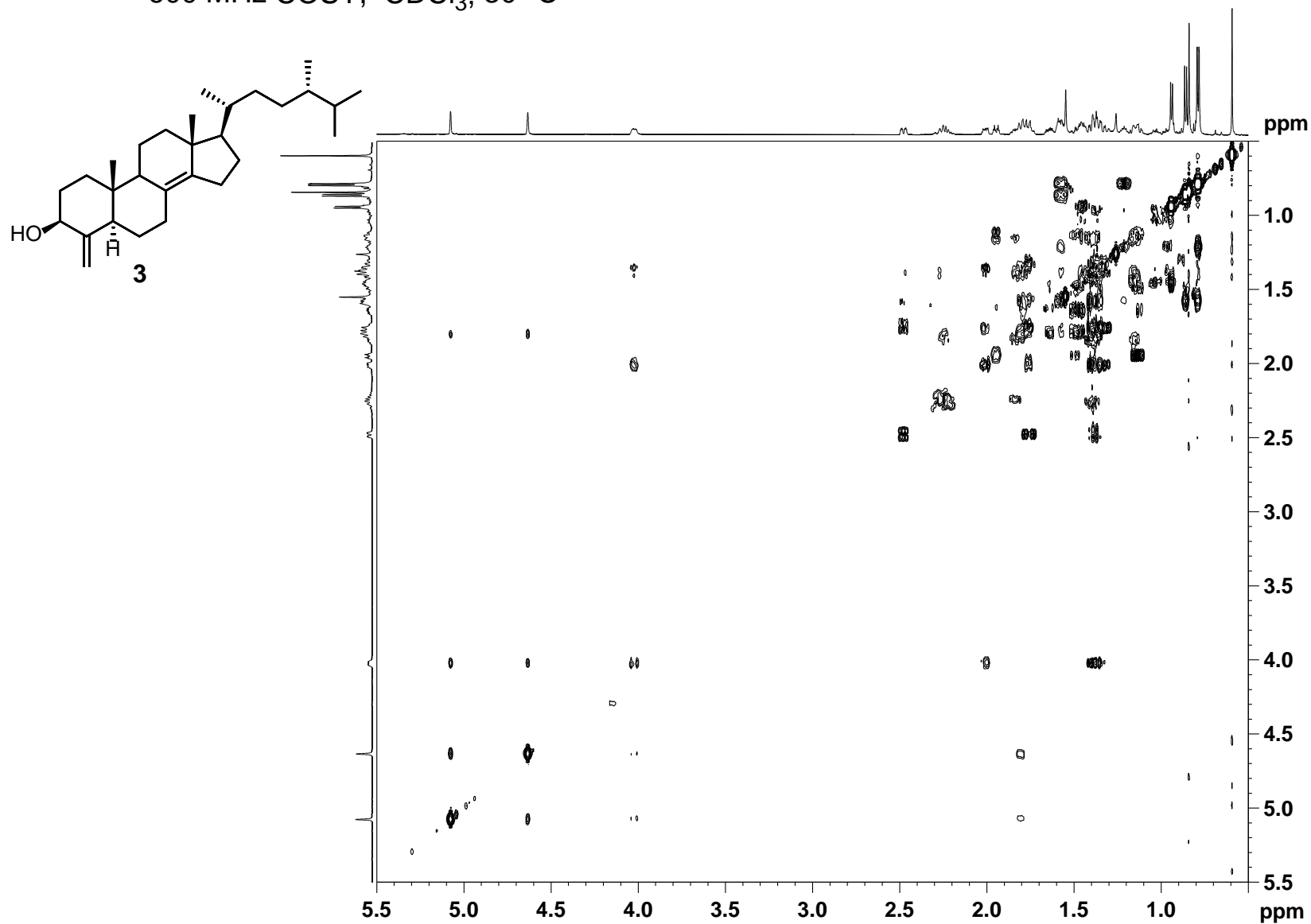
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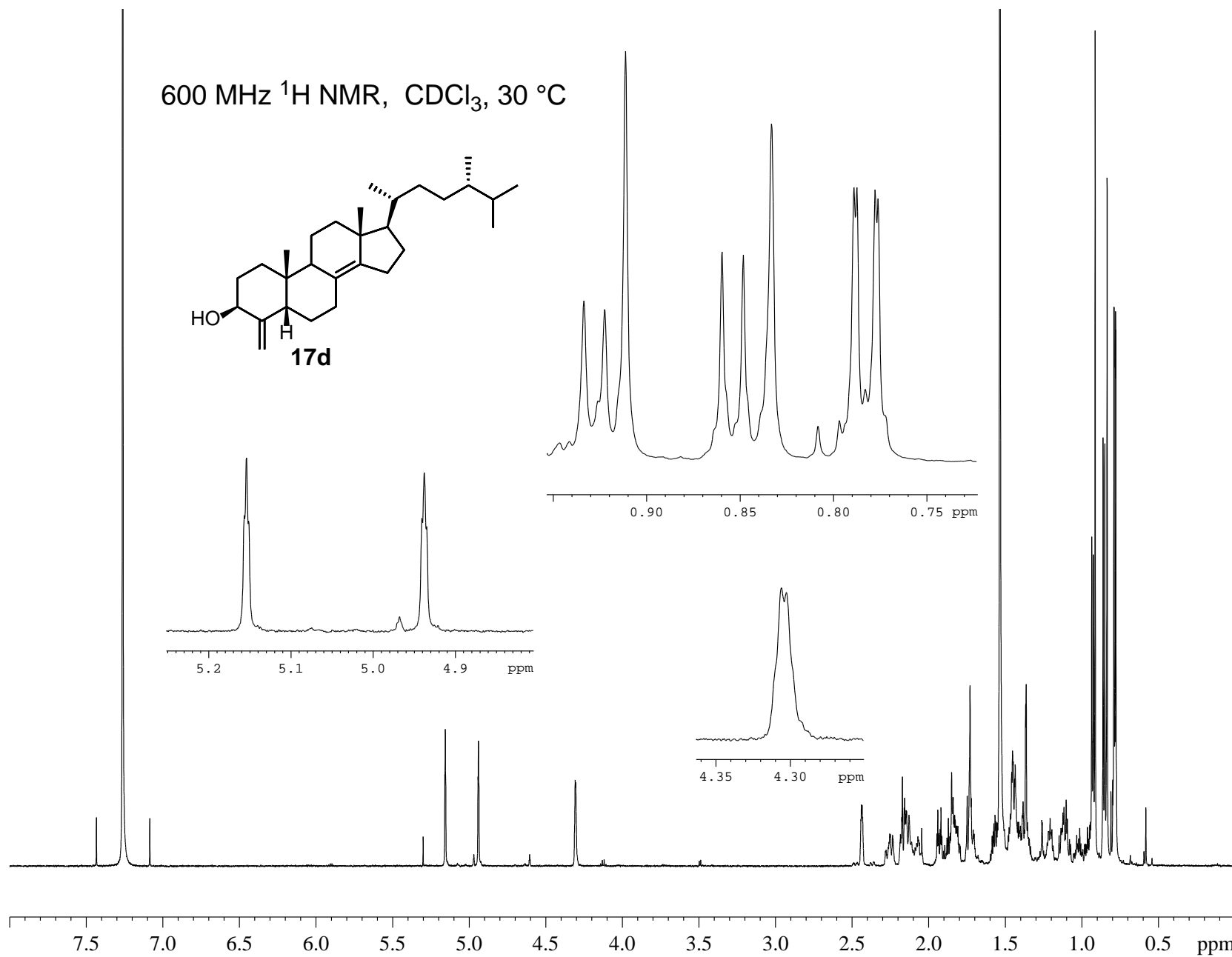
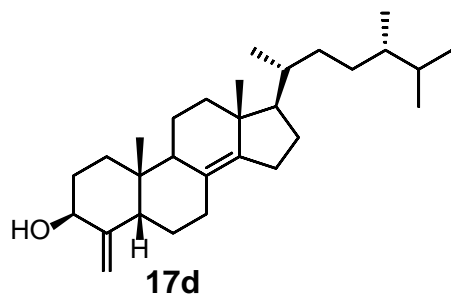
600 MHz HMBC, CDCl₃, 30 °C



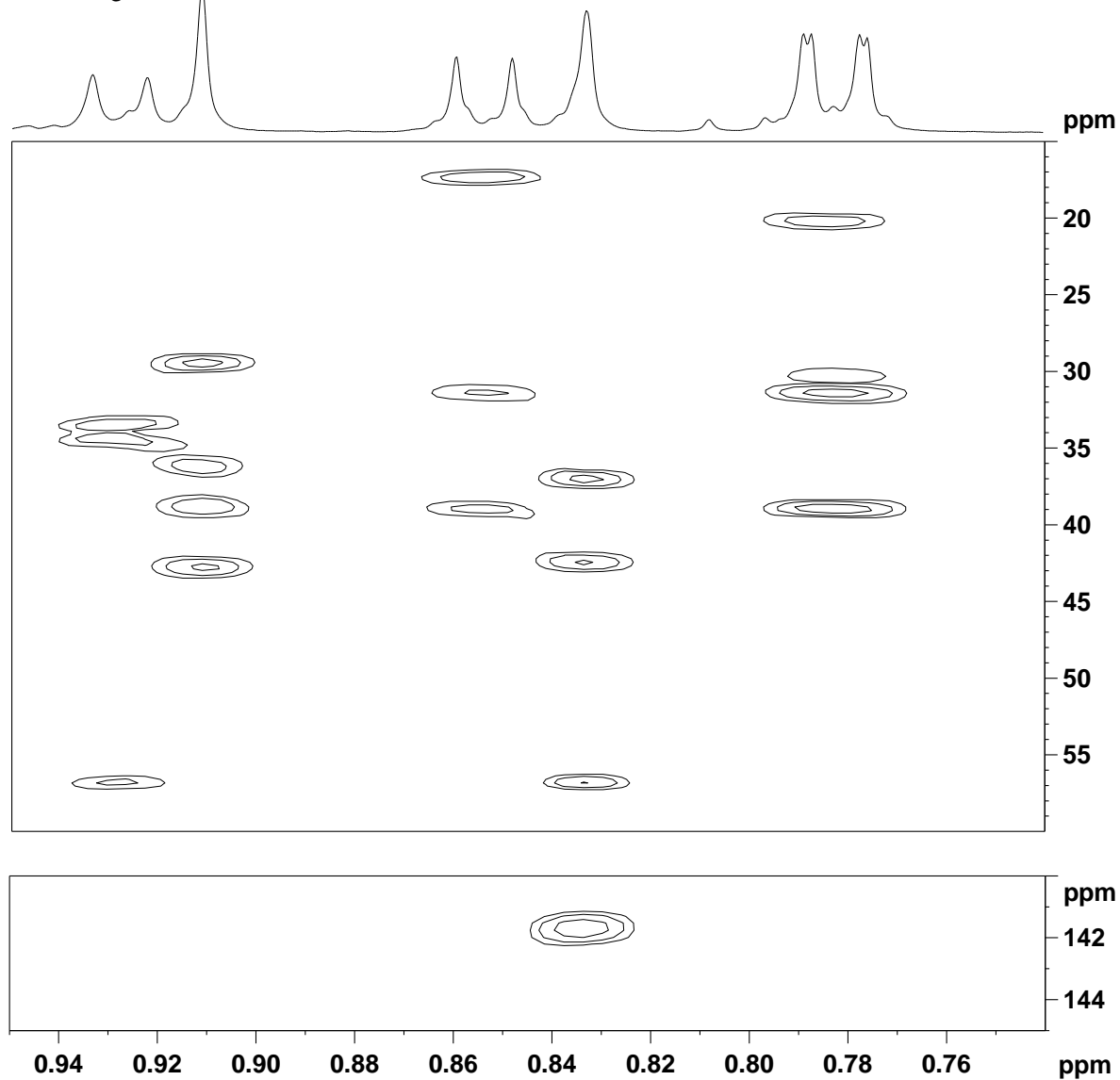
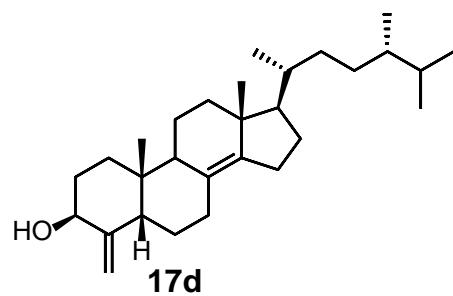
600 MHz COSY, CDCl_3 , 30 °C



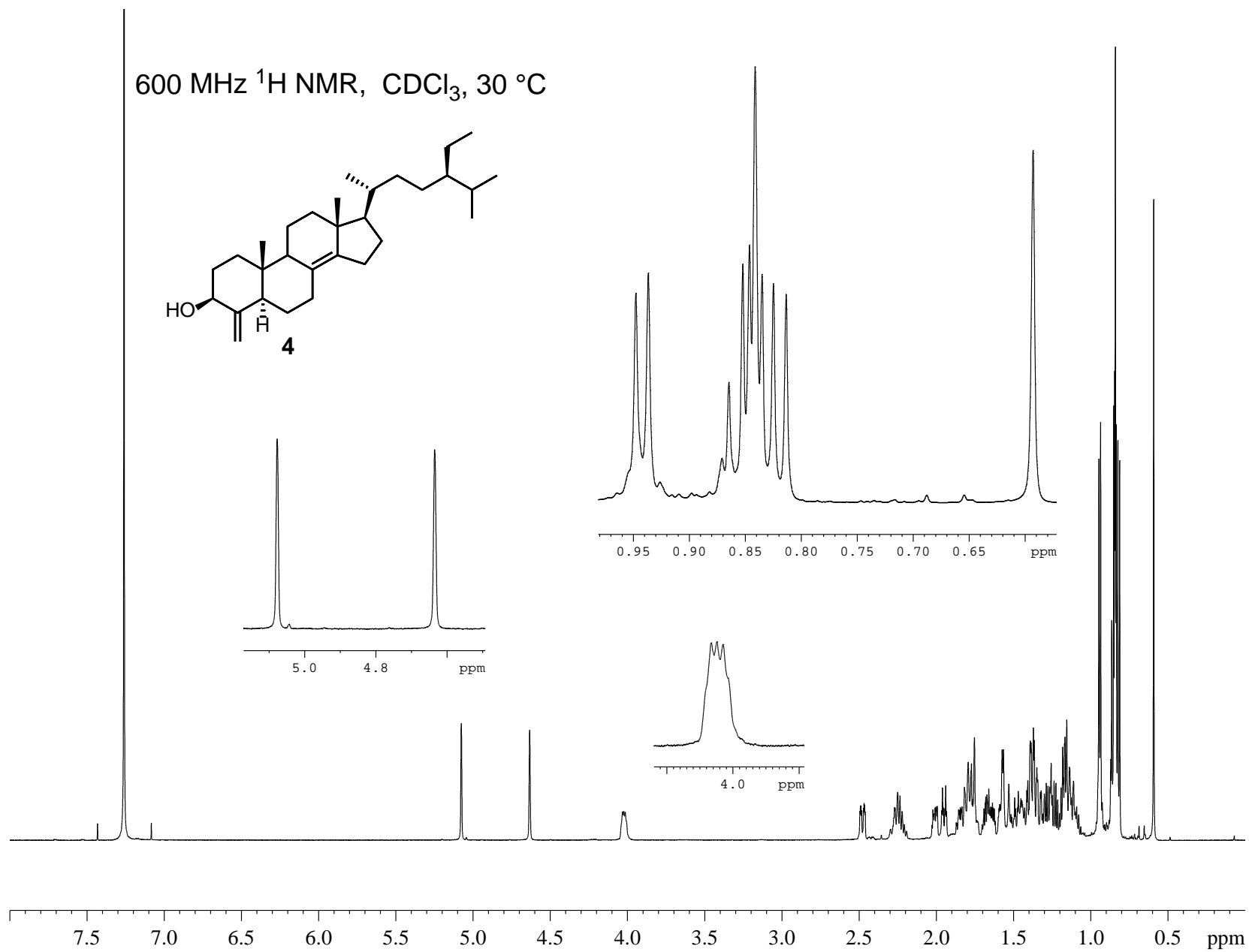
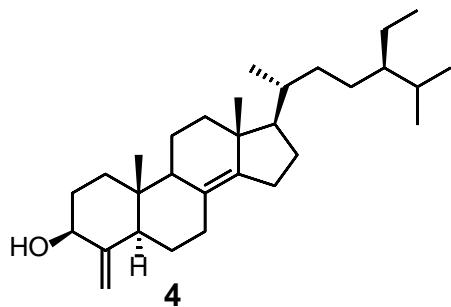
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



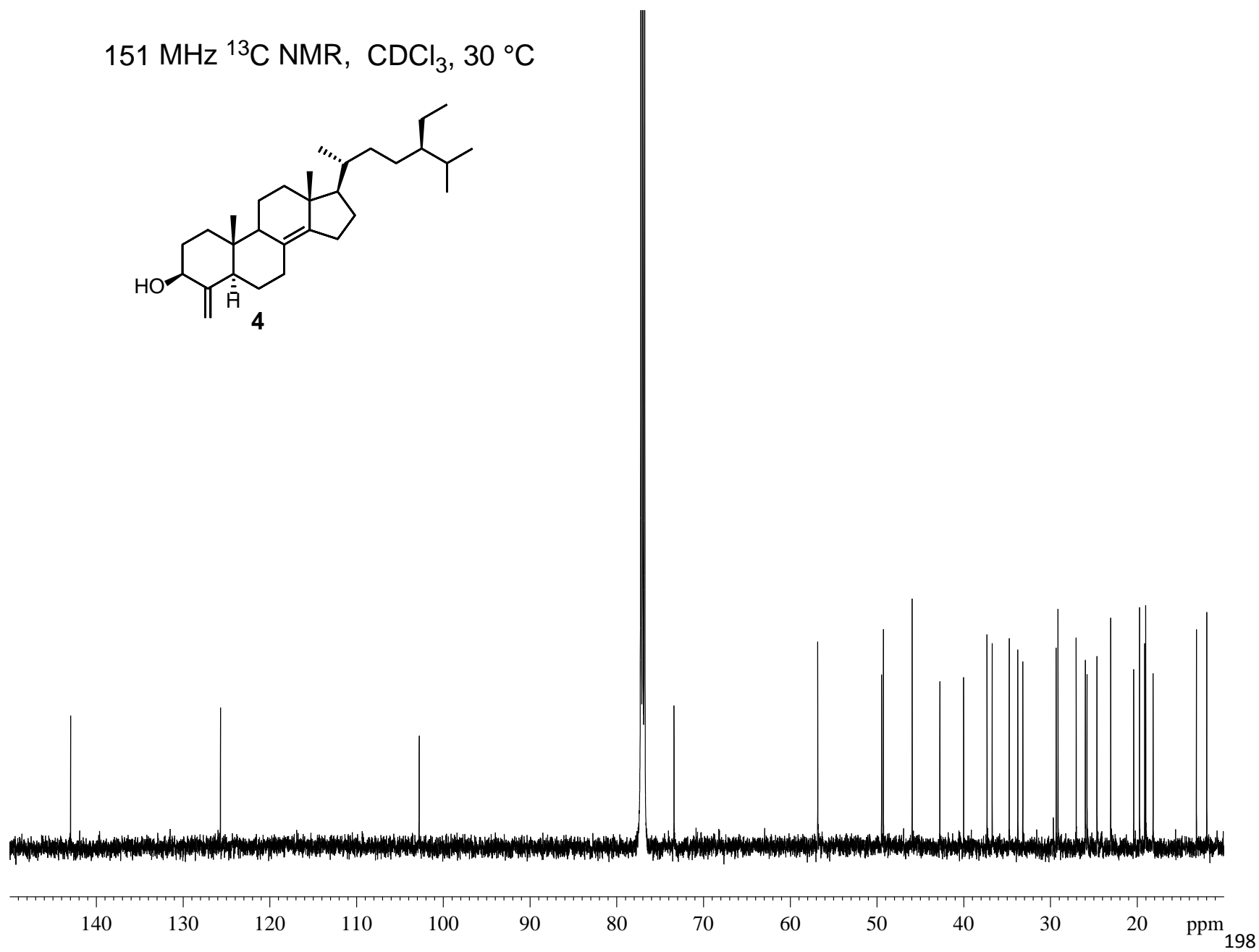
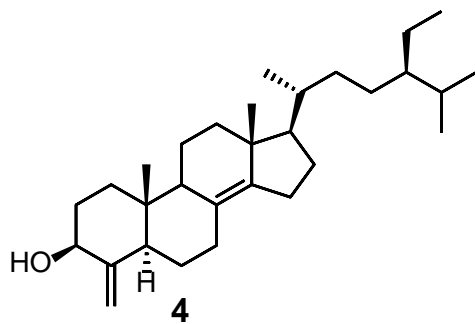
600 MHz HMBC, CDCl₃, 30 °C



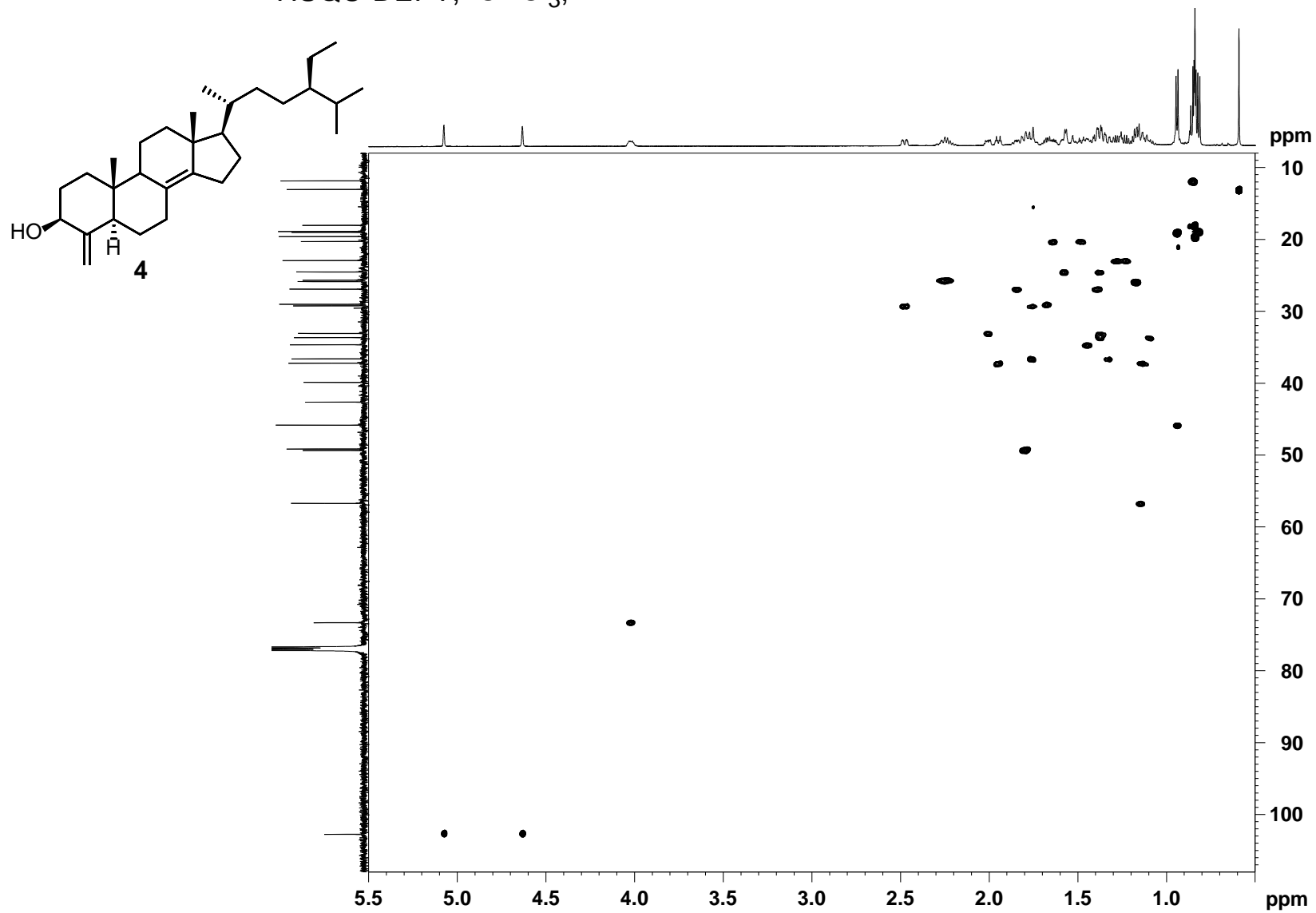
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



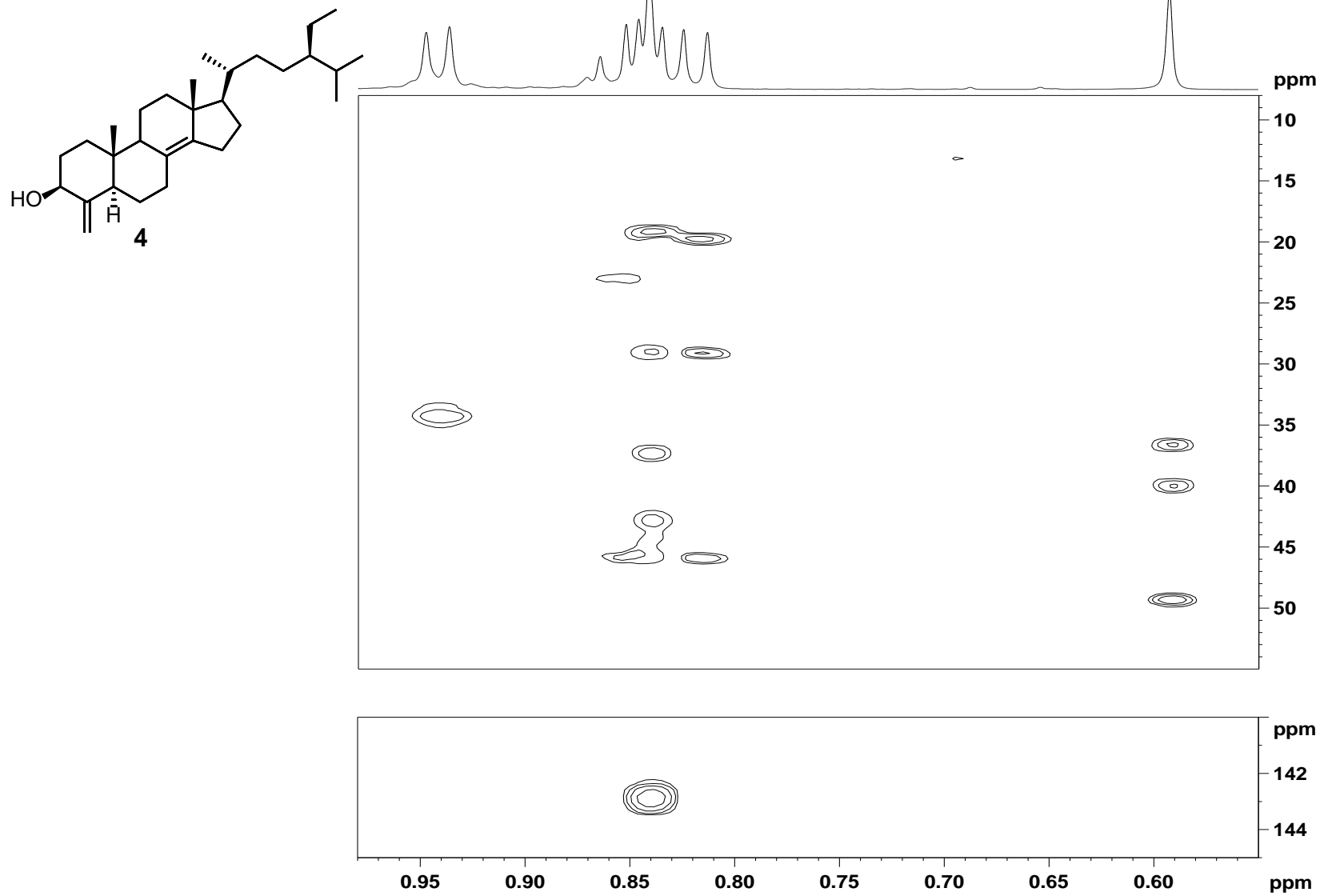
151 MHz ^{13}C NMR, CDCl_3 , 30 °C



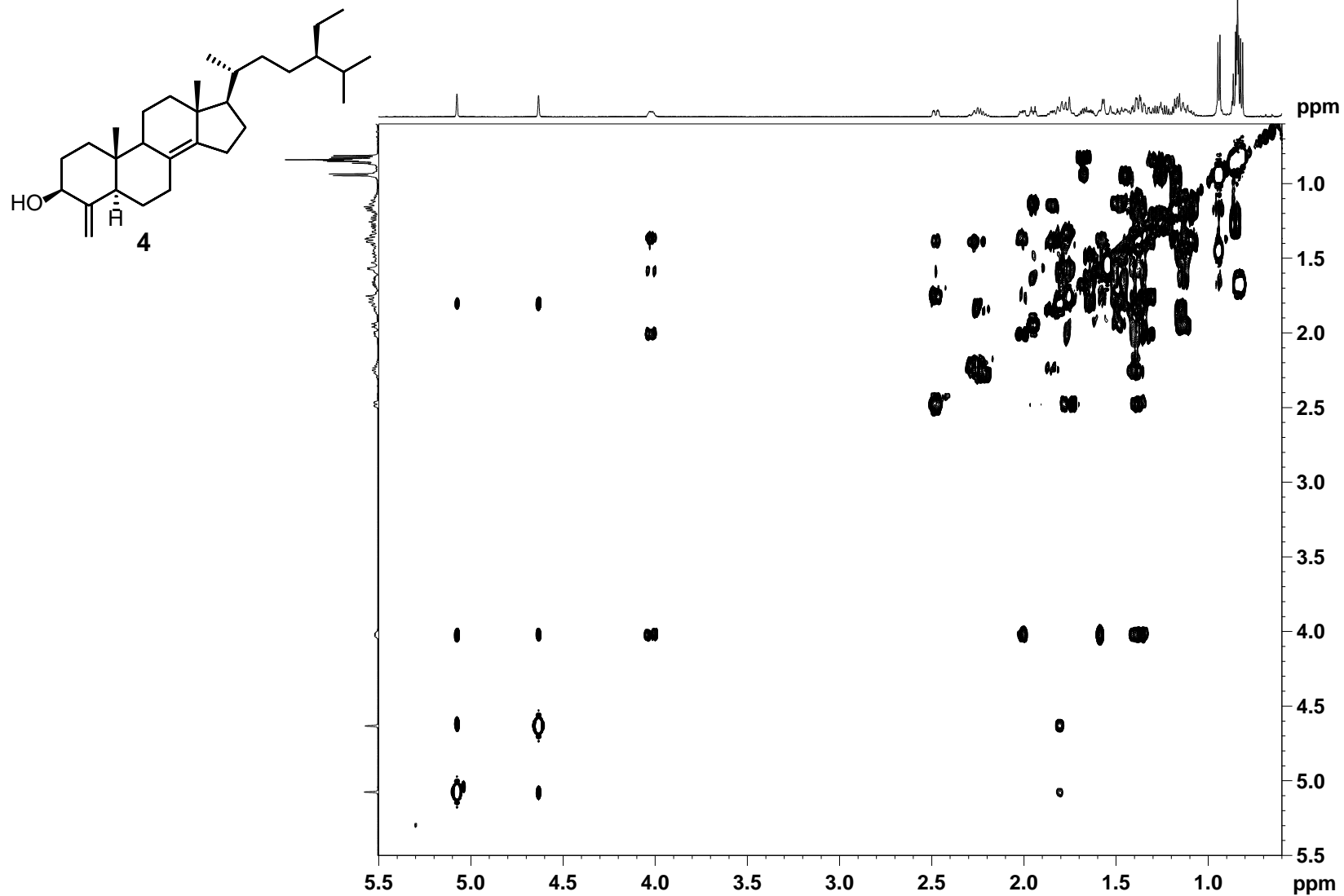
600 MHz HSQC-DEPT, CDCl_3 , 30 °C



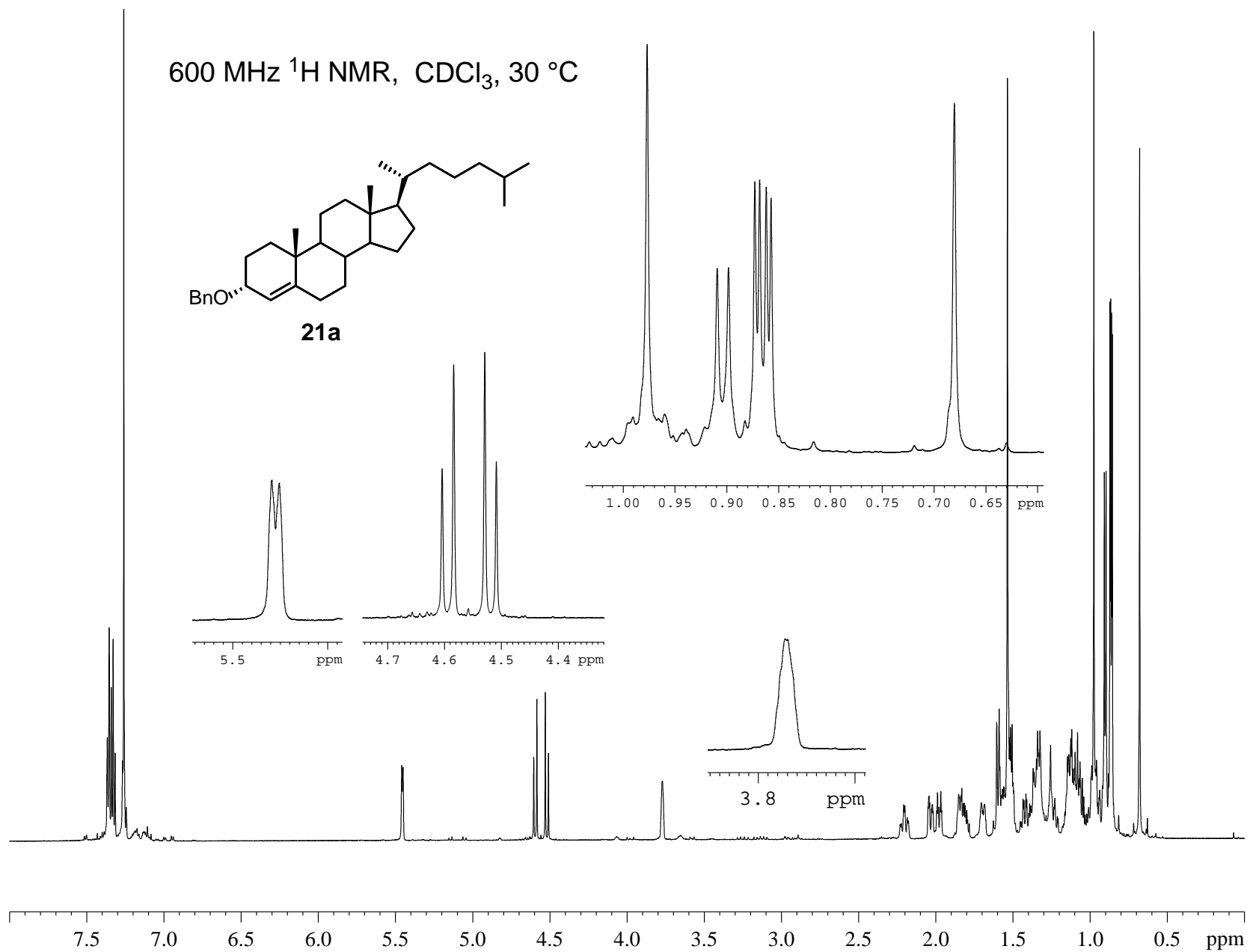
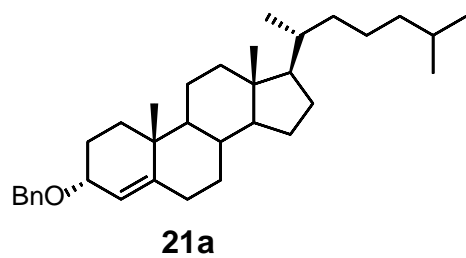
600 MHz HMBC, CDCl₃, 30 °C



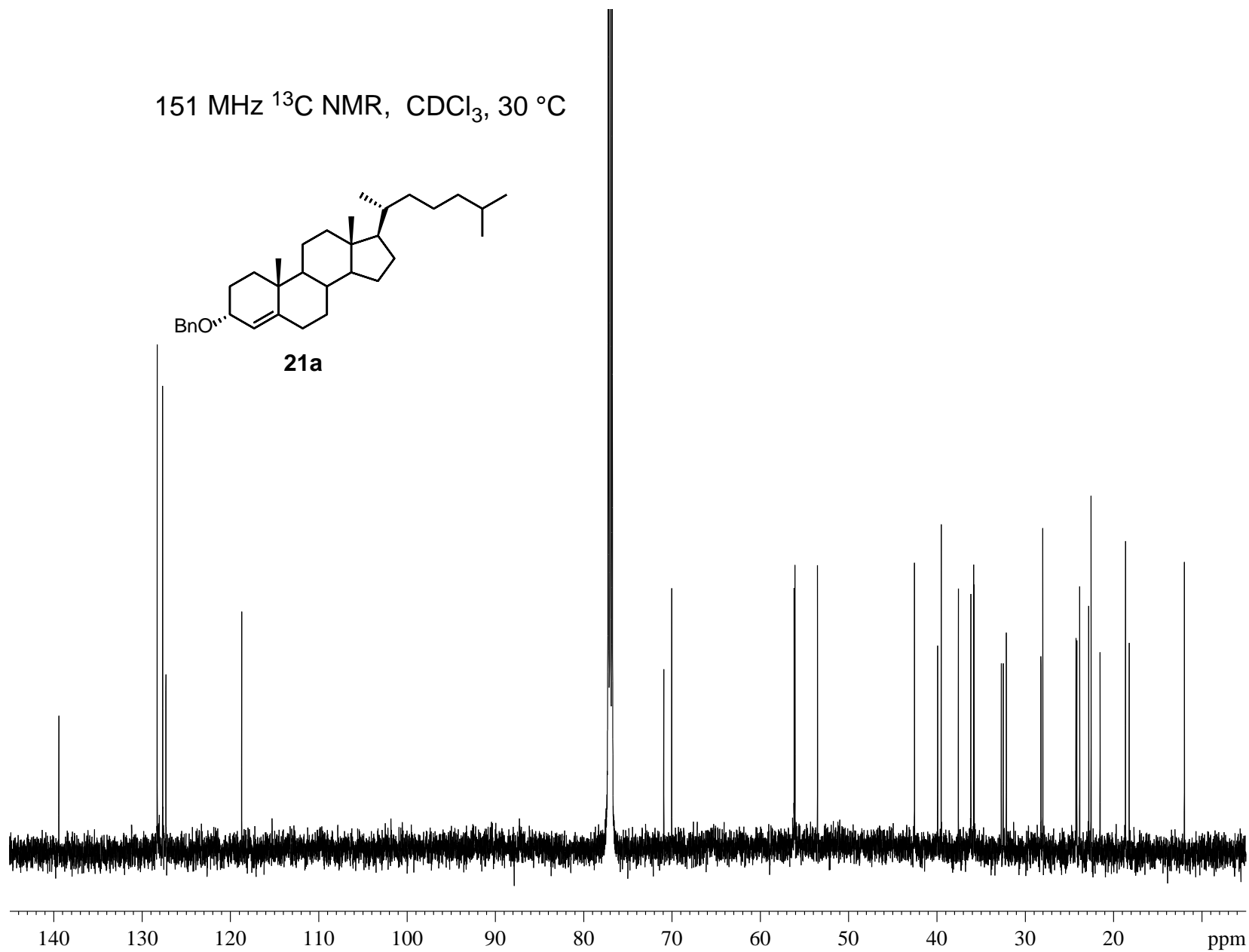
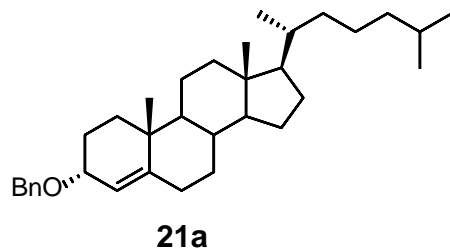
600 MHz COSY, CDCl_3 , 30 °C

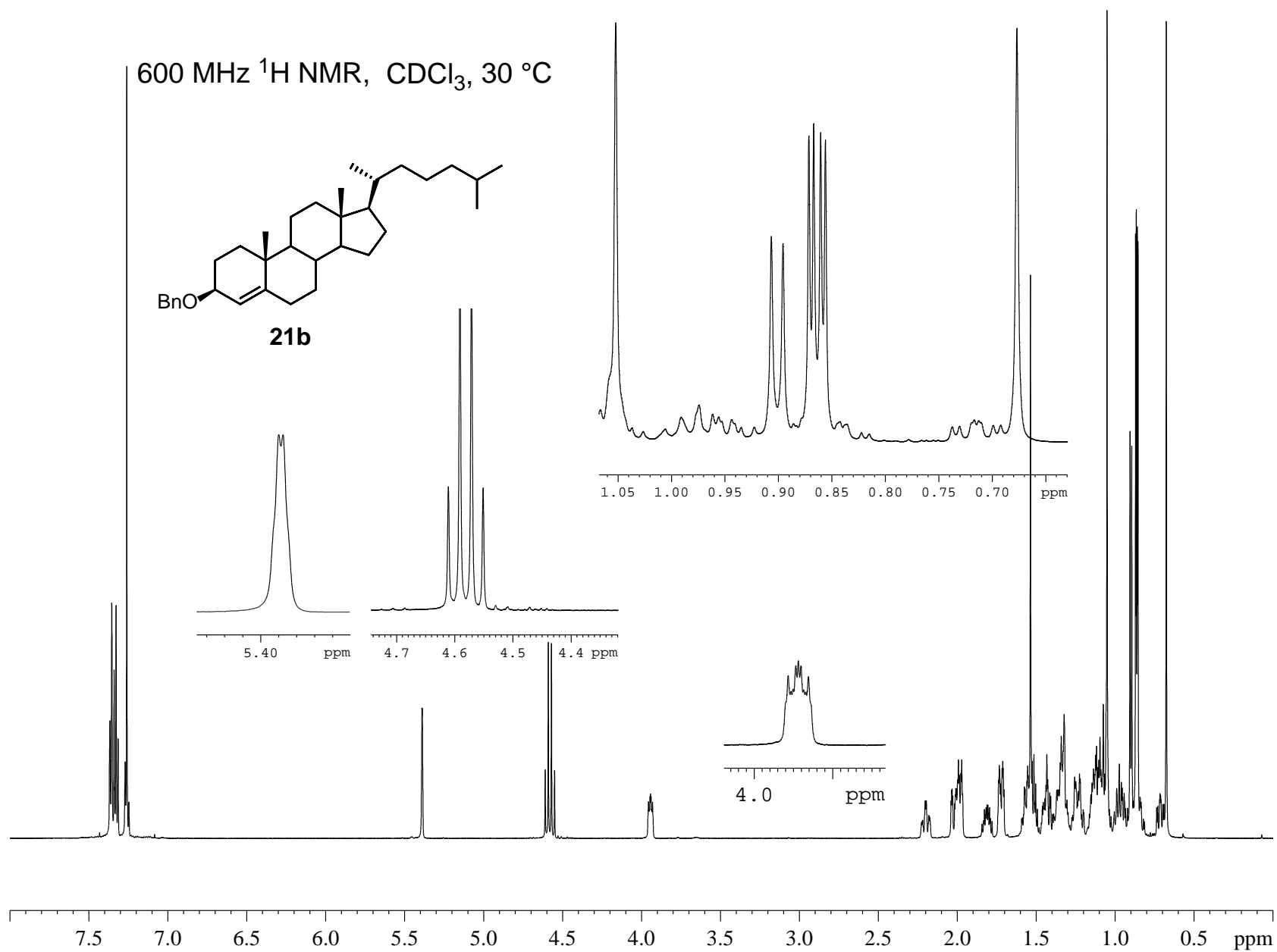


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

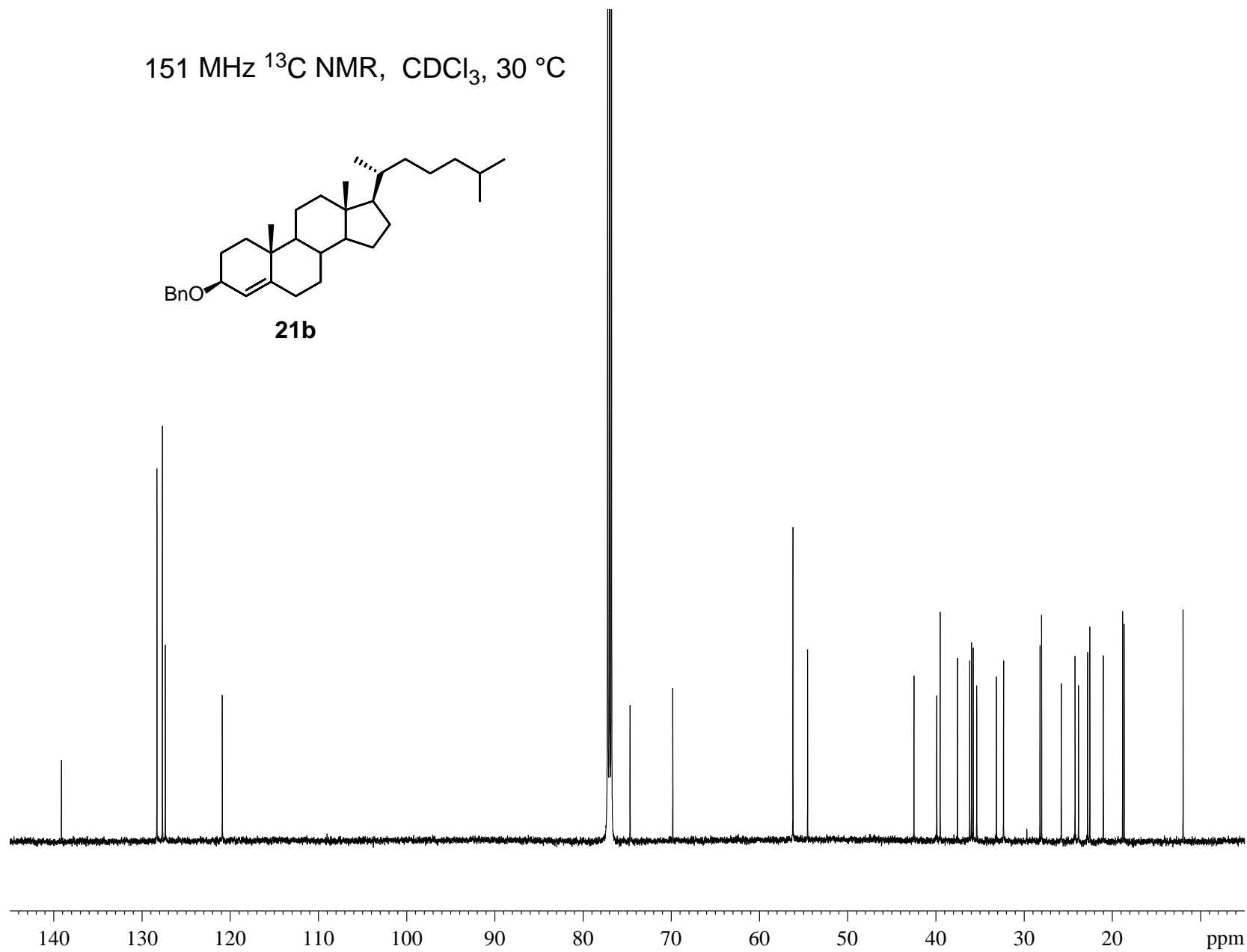
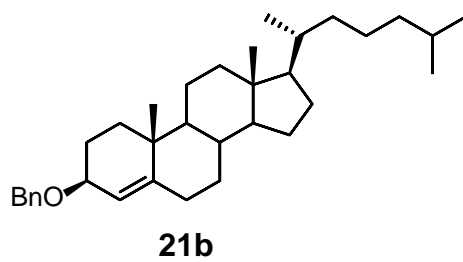


151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$

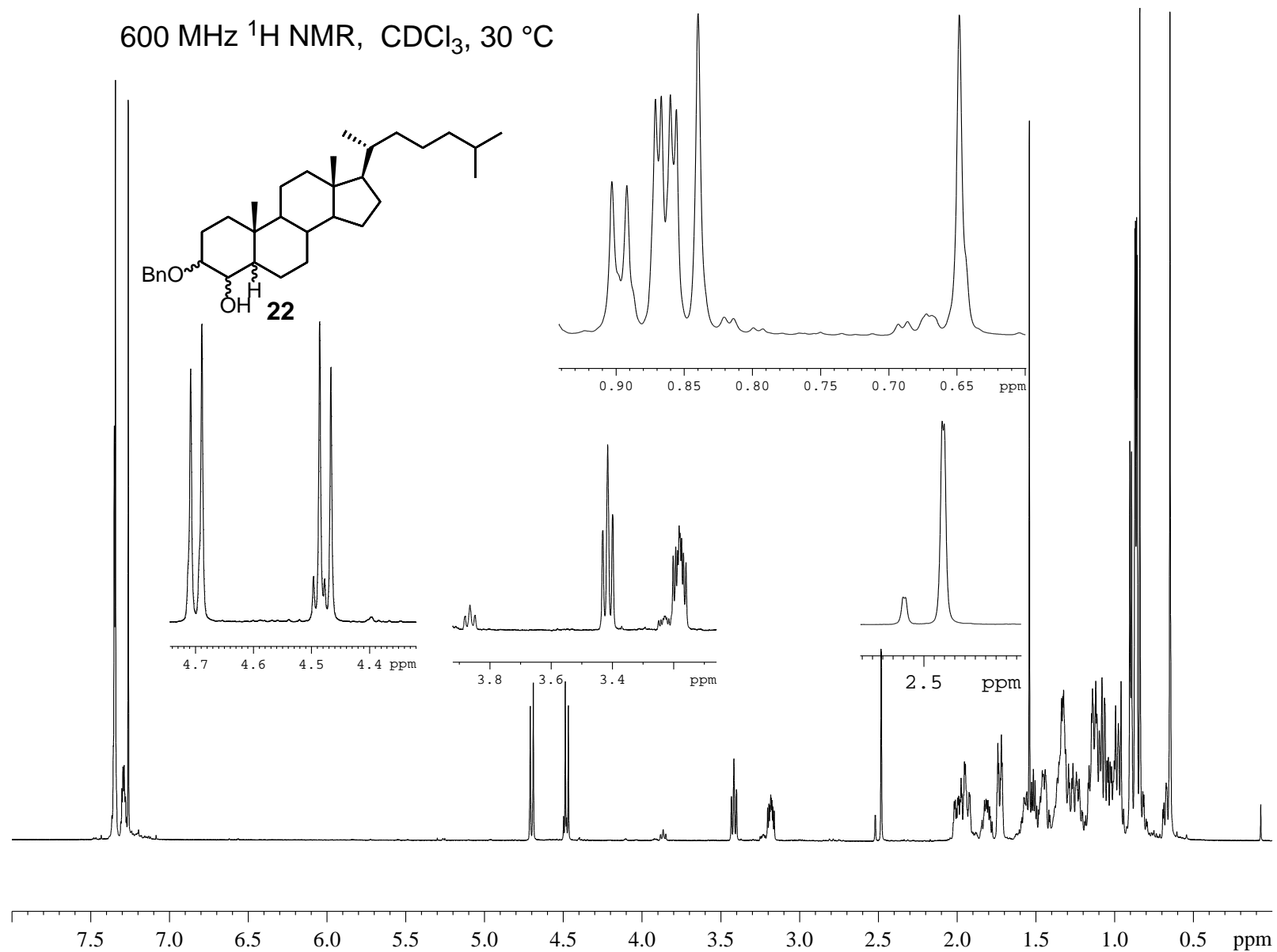




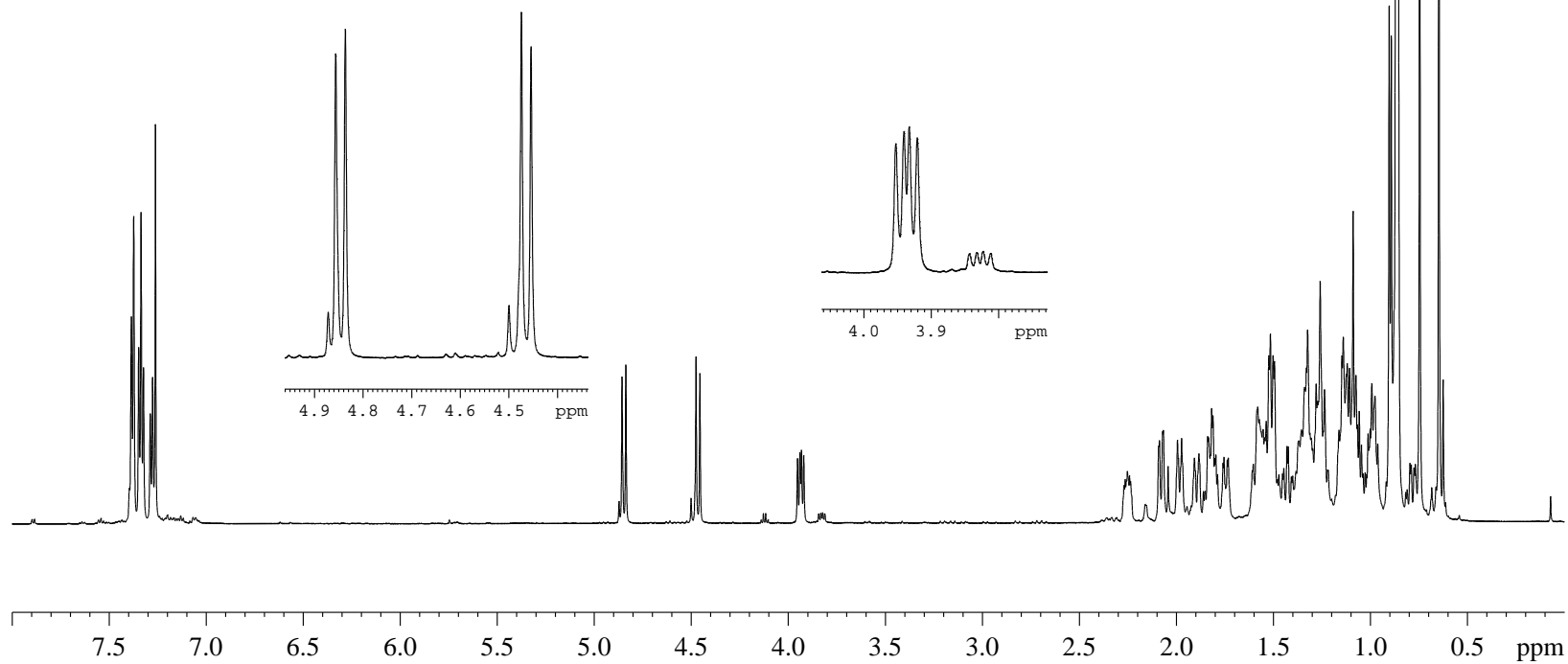
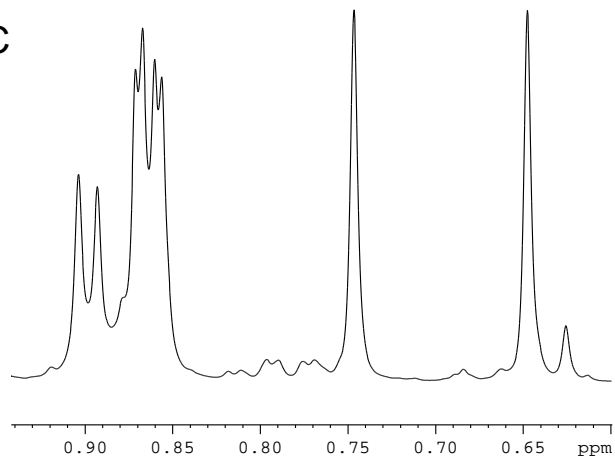
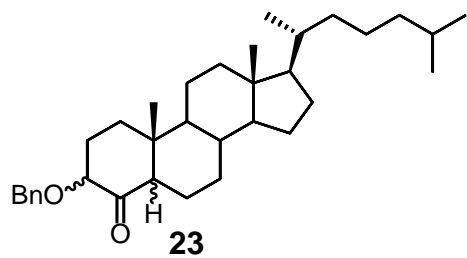
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



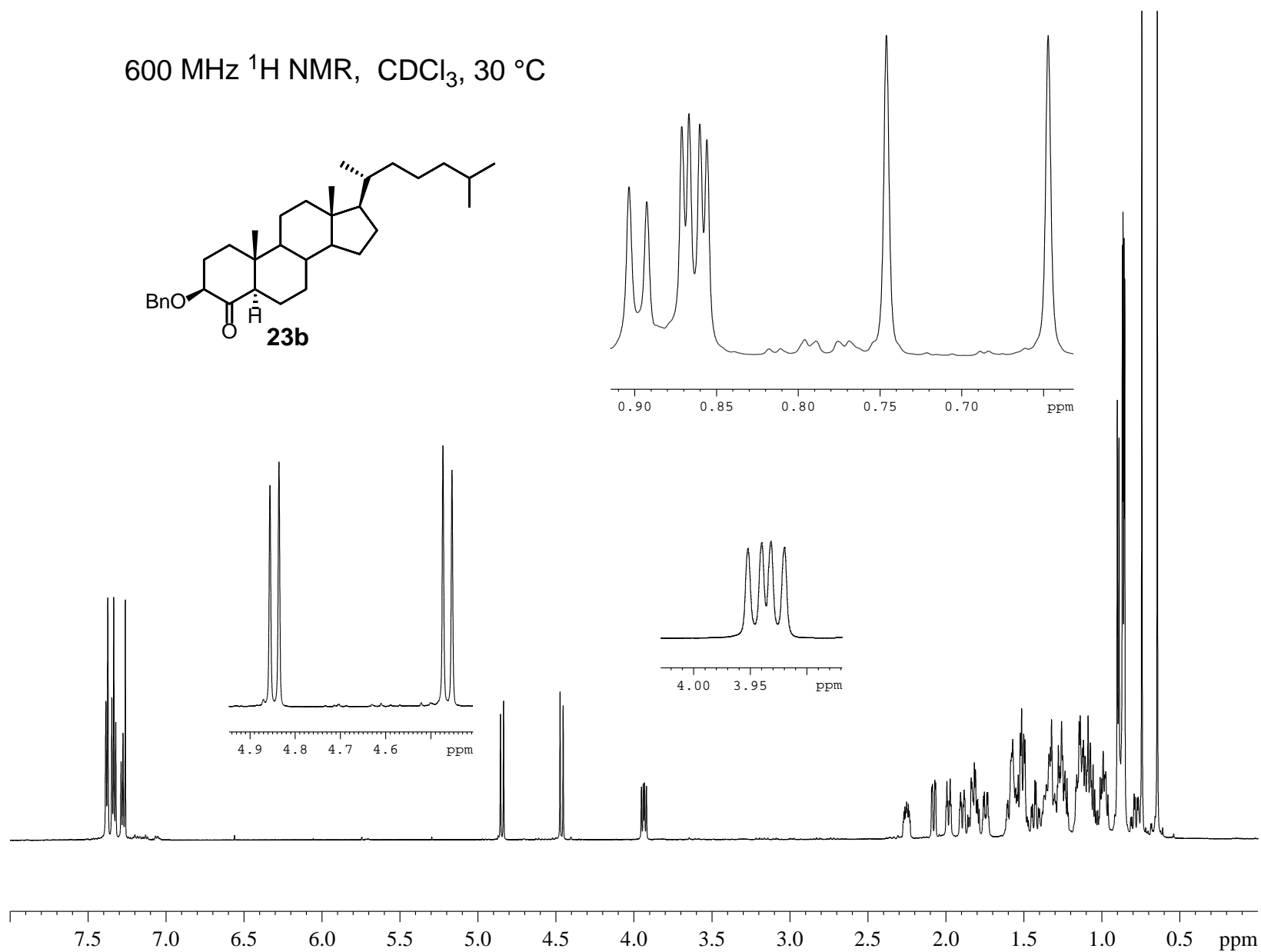
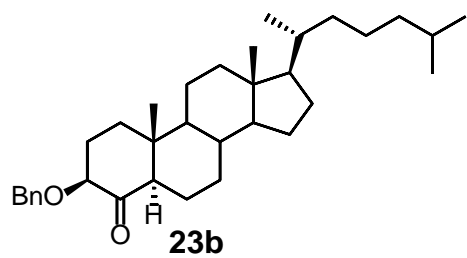
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



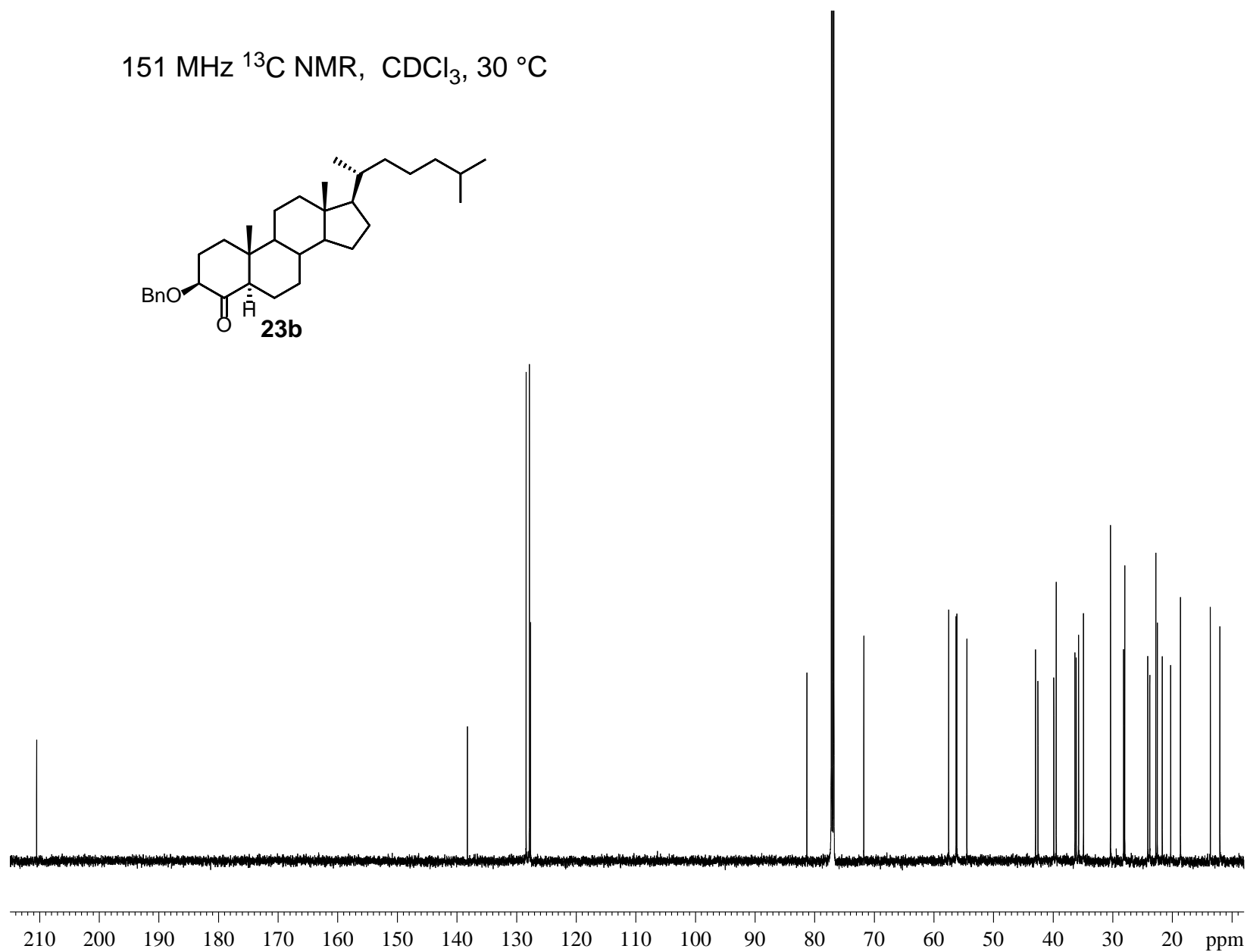
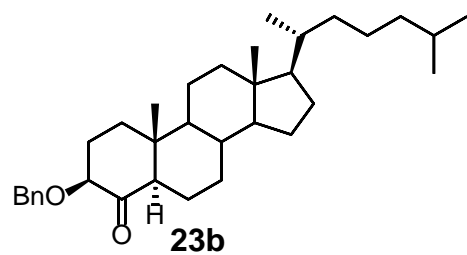
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



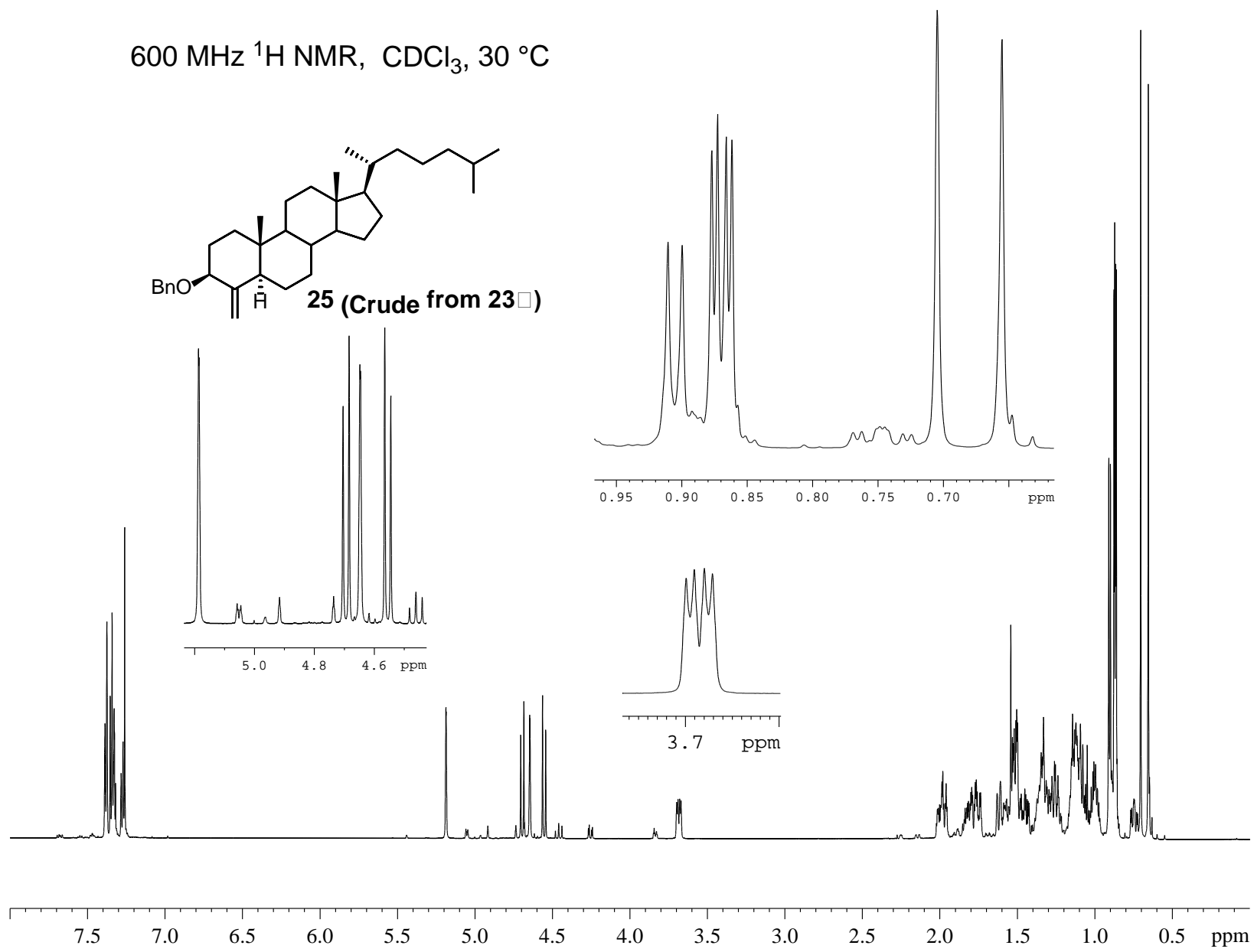
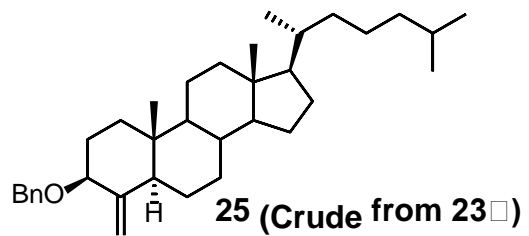
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



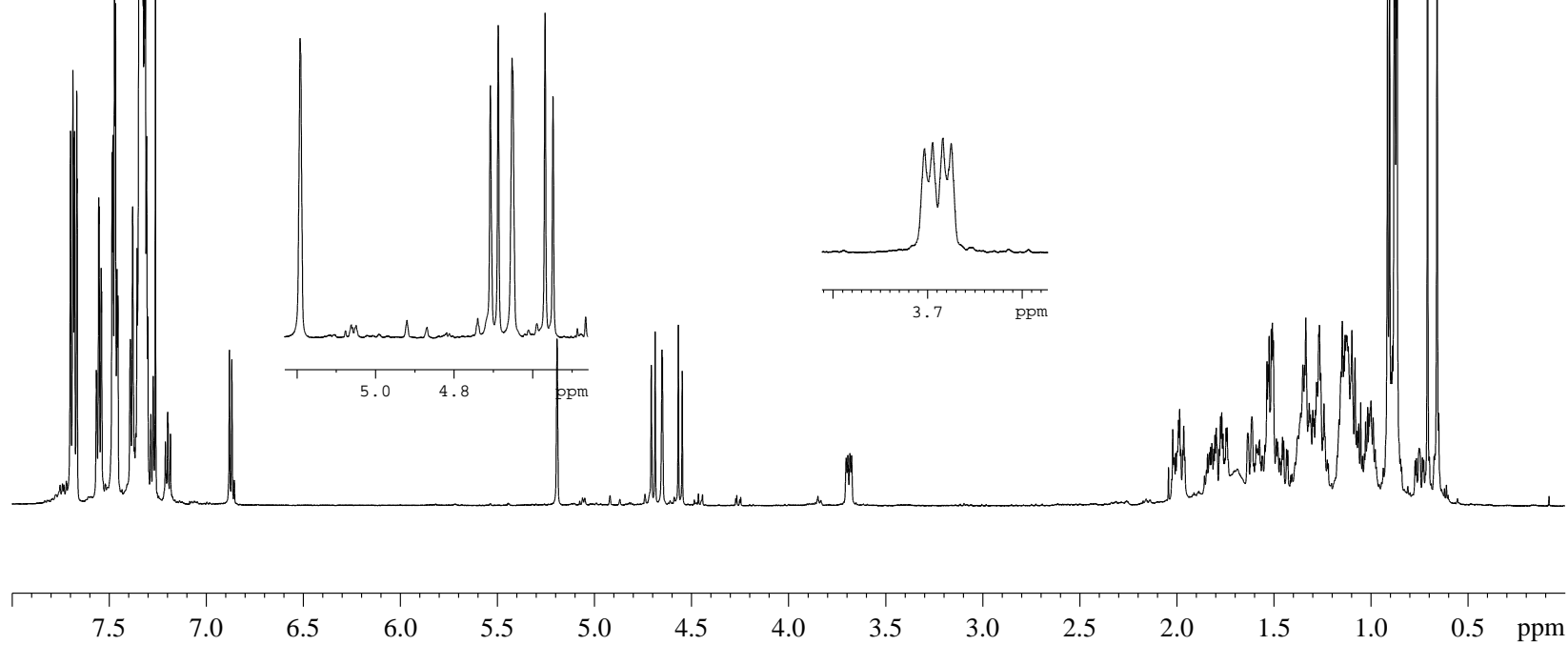
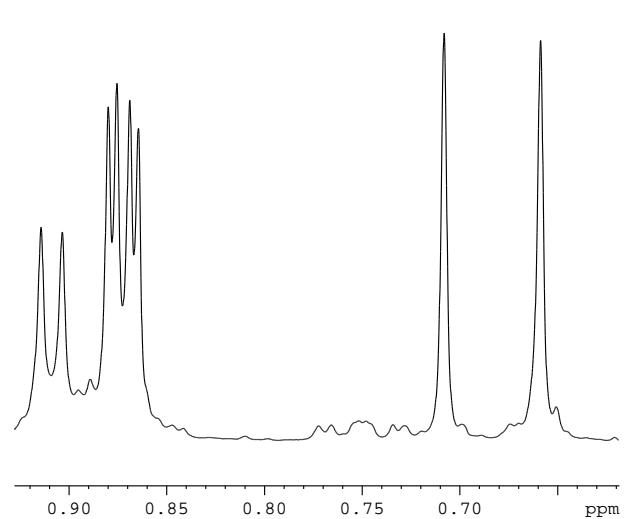
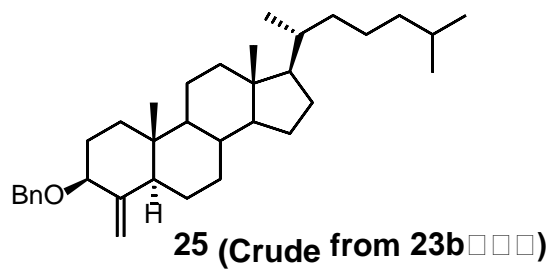
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



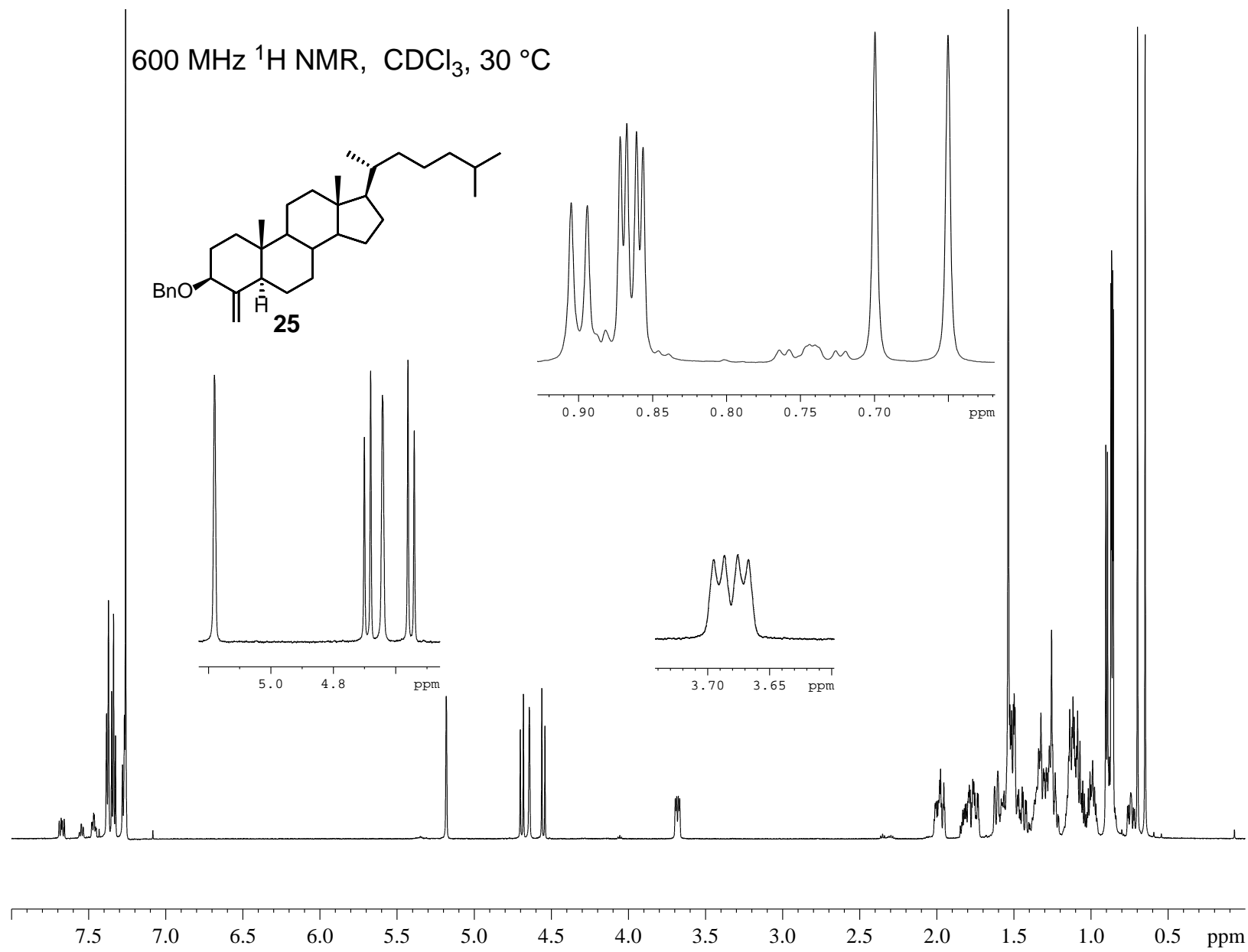
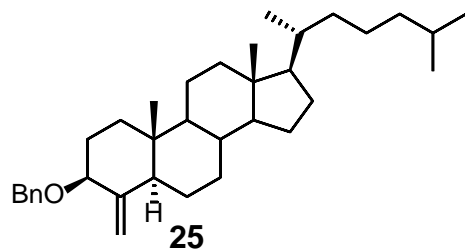
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



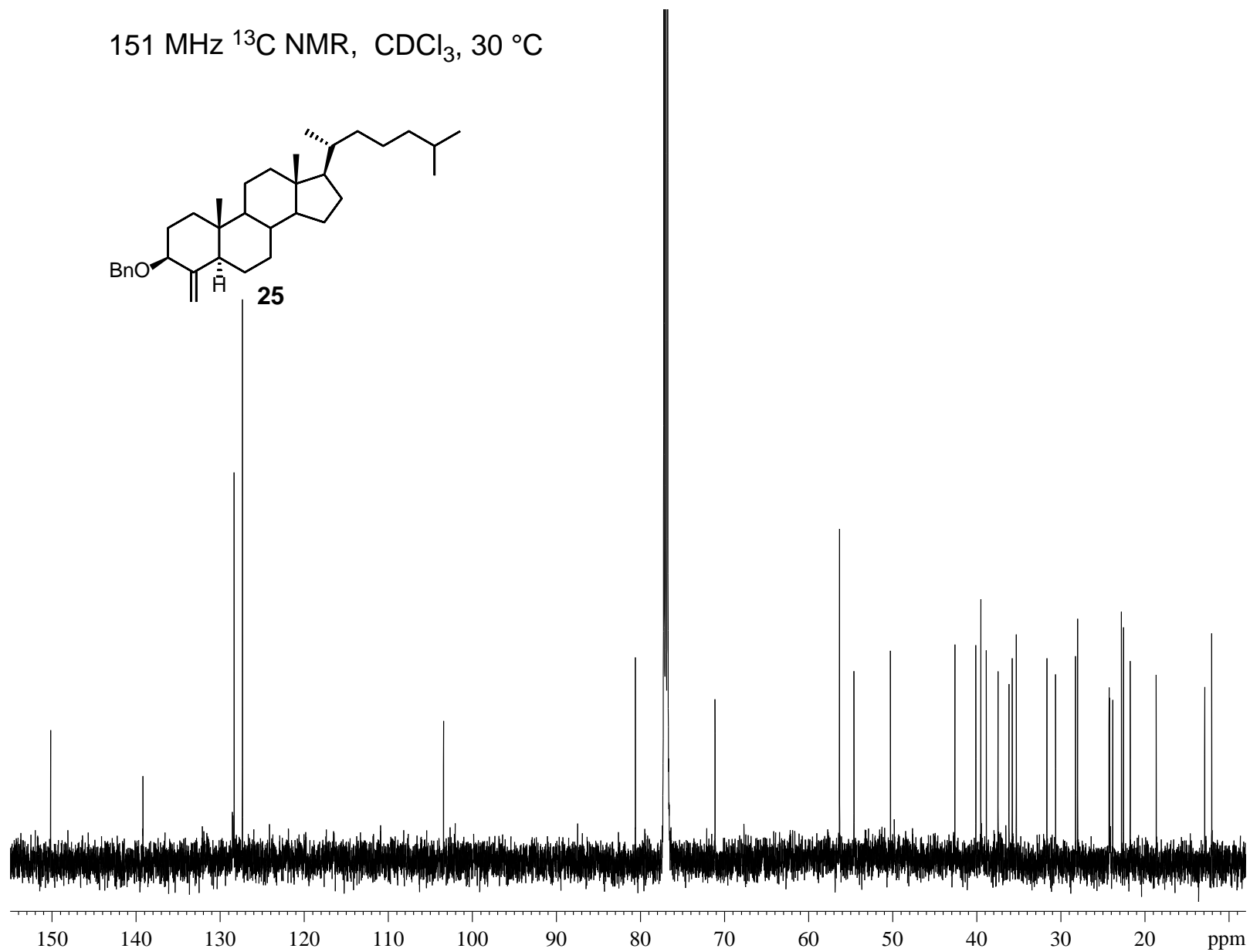
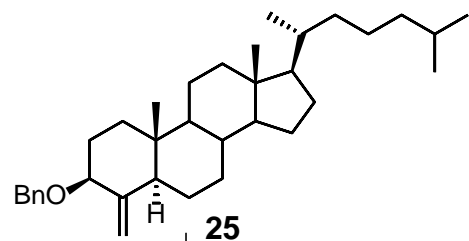
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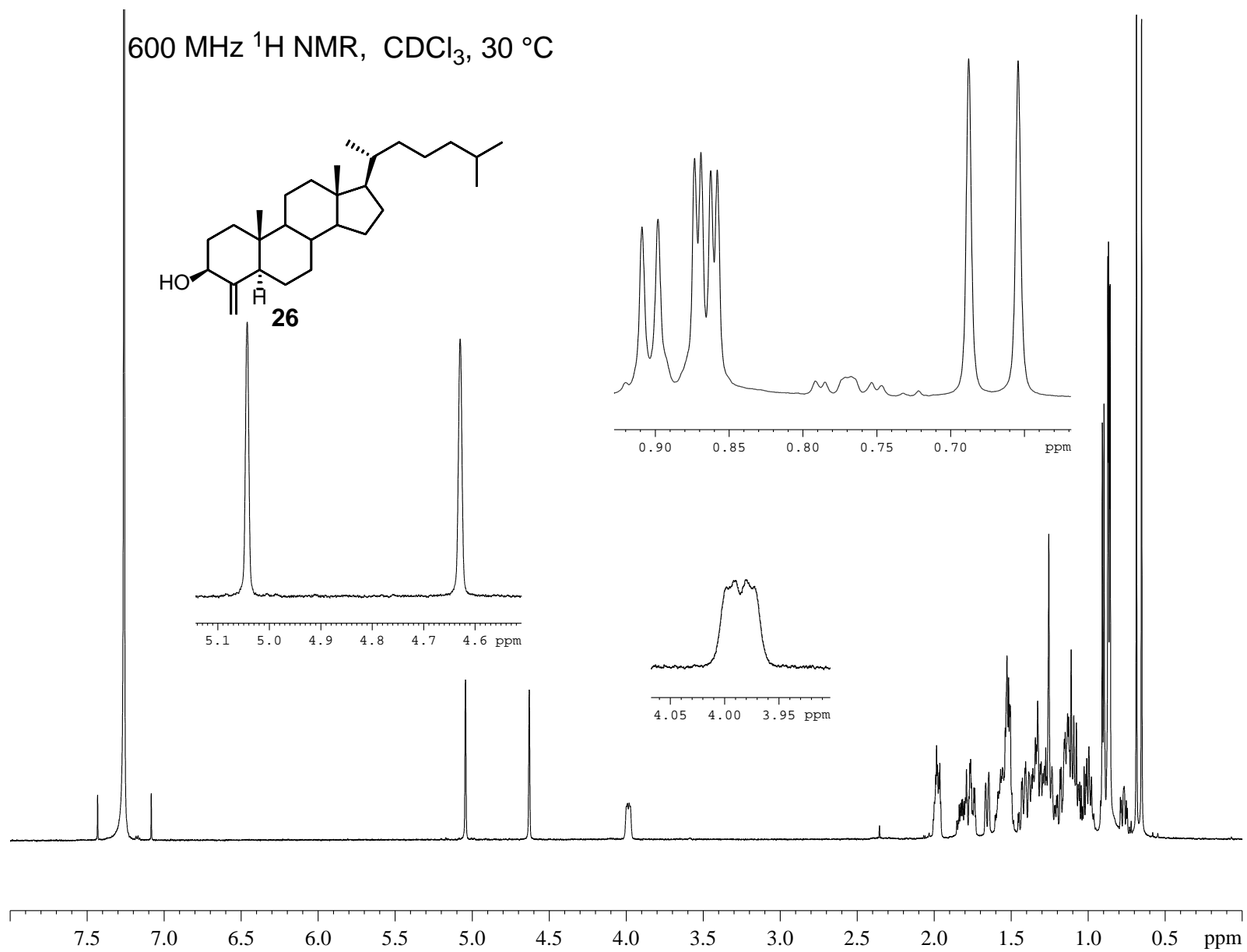
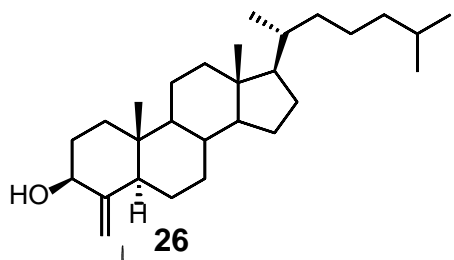
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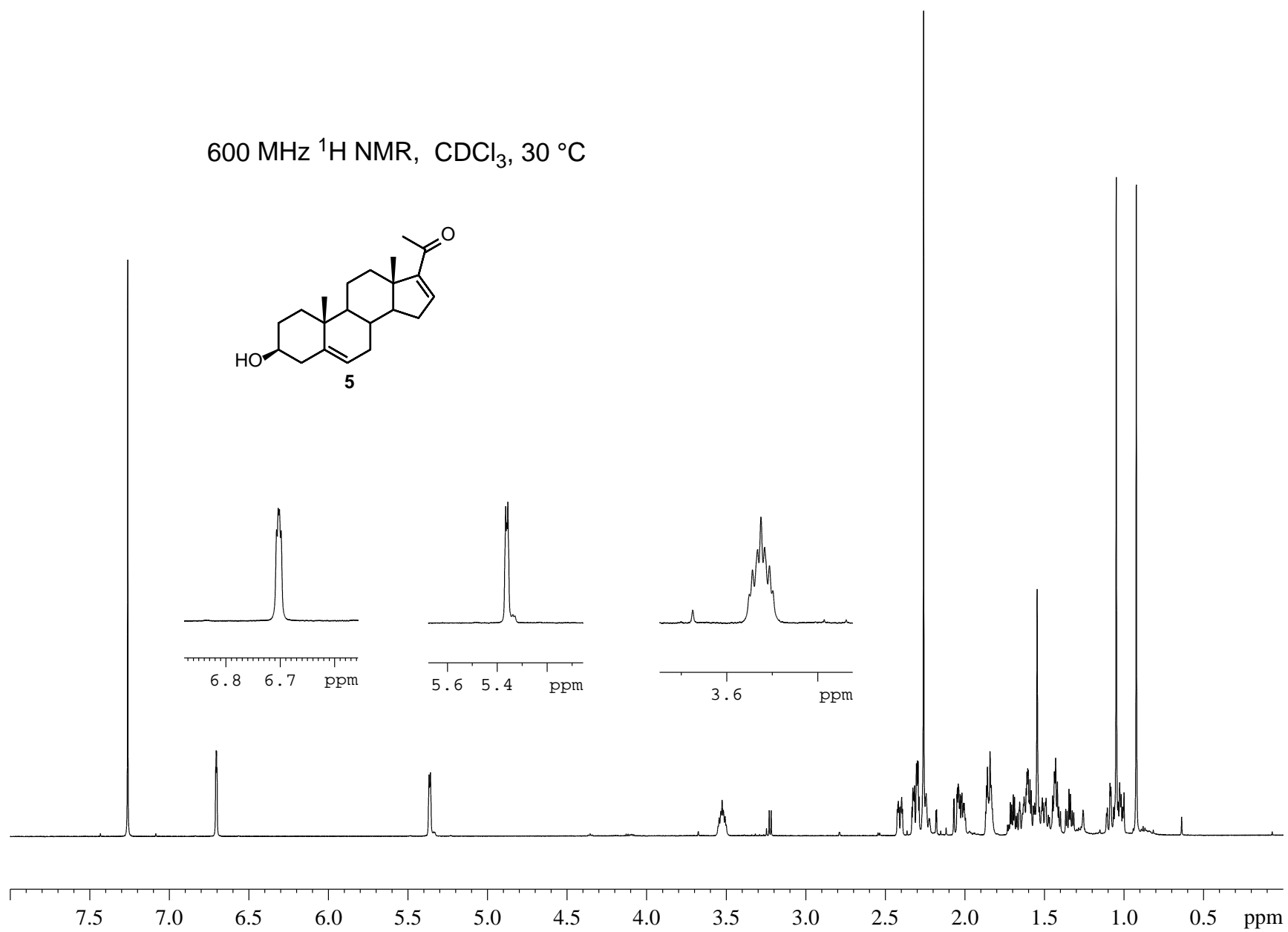
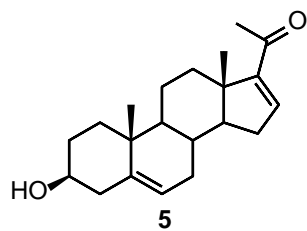
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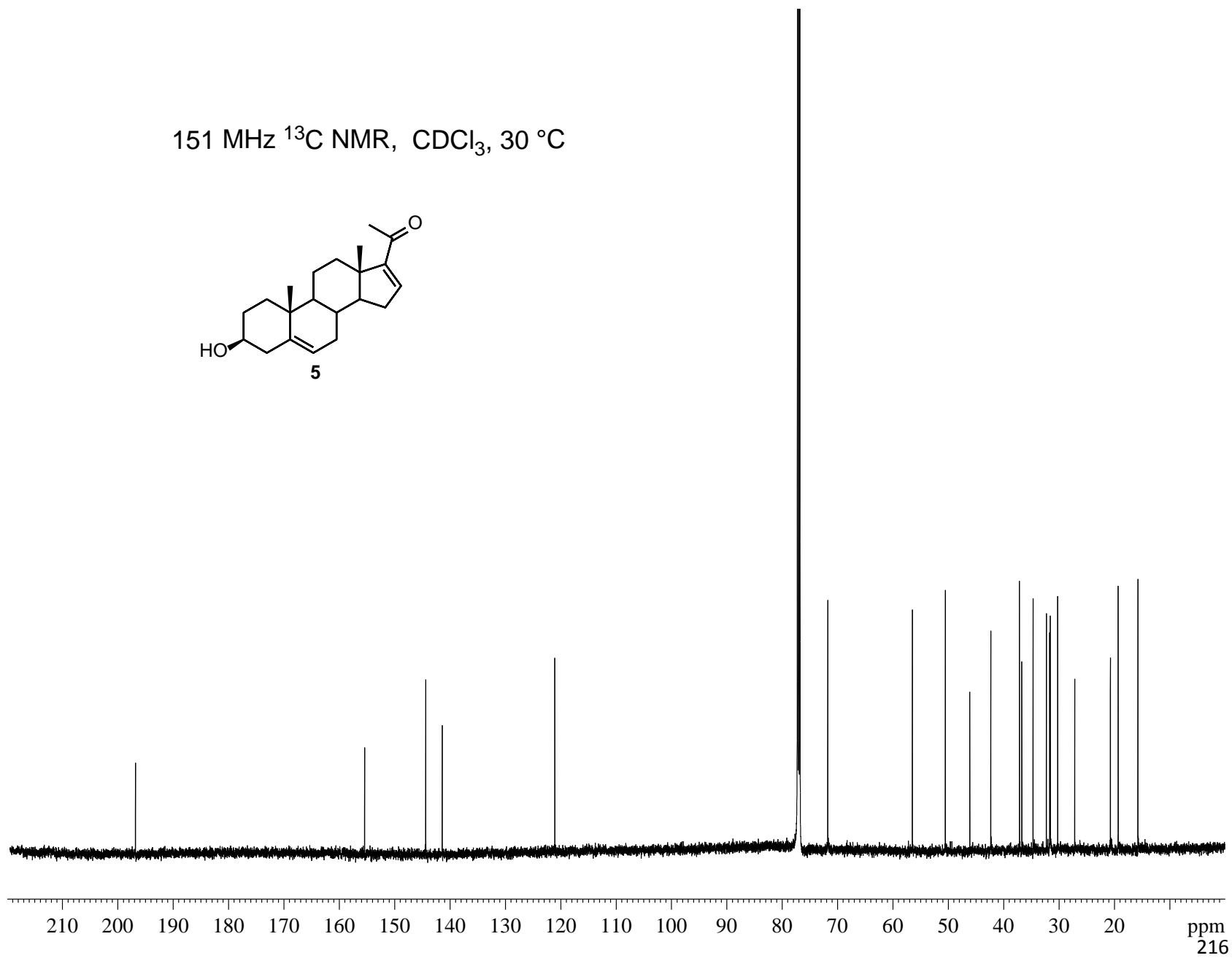
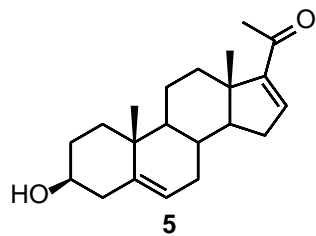
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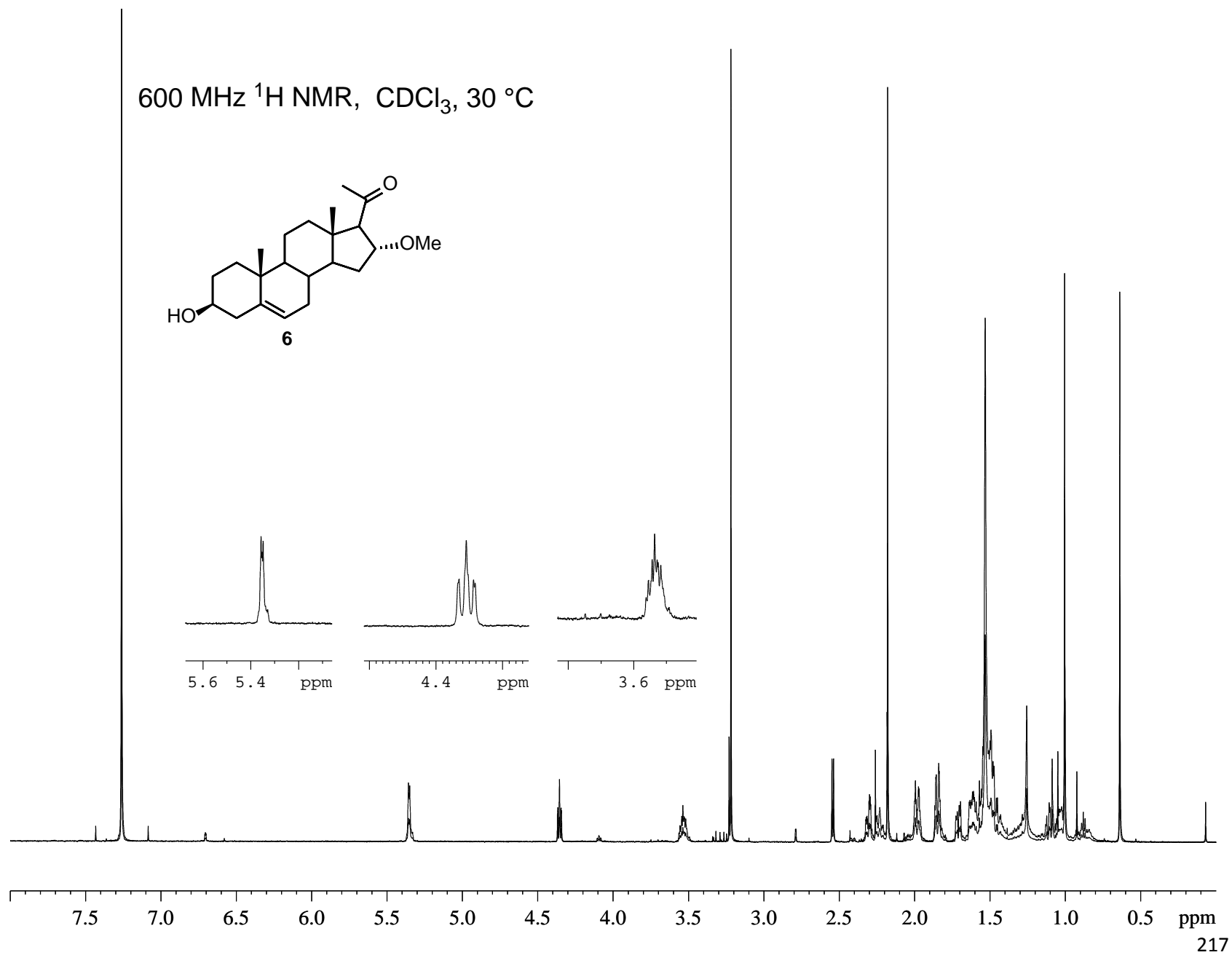
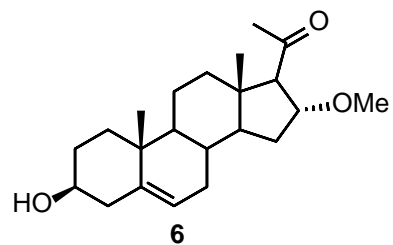
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



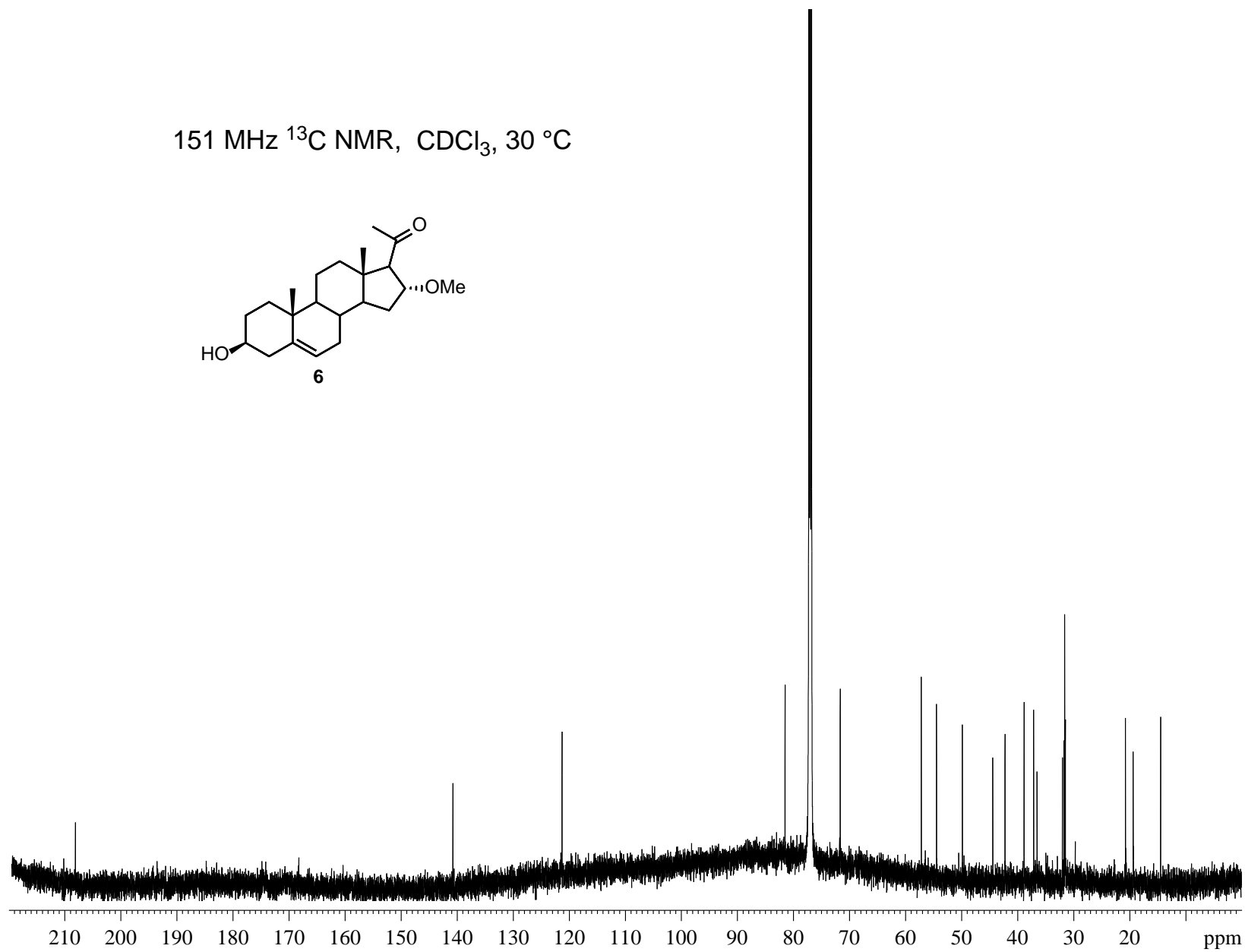
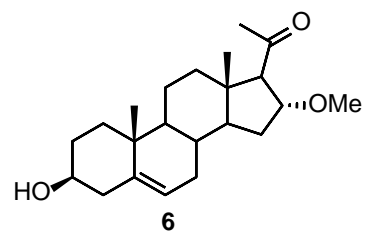
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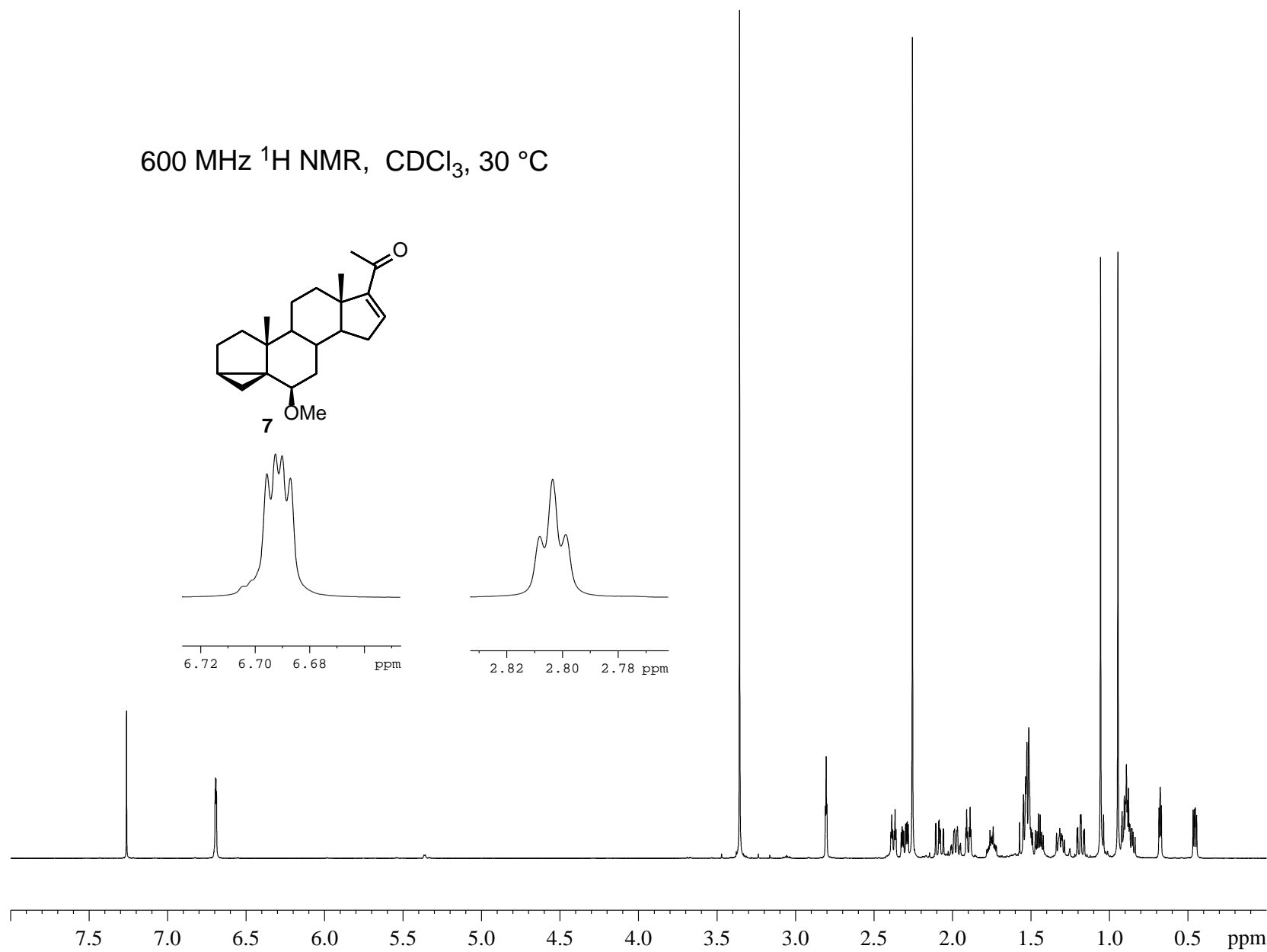
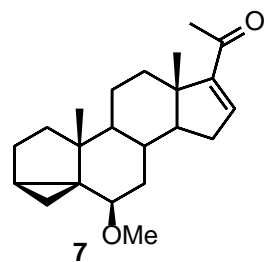
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



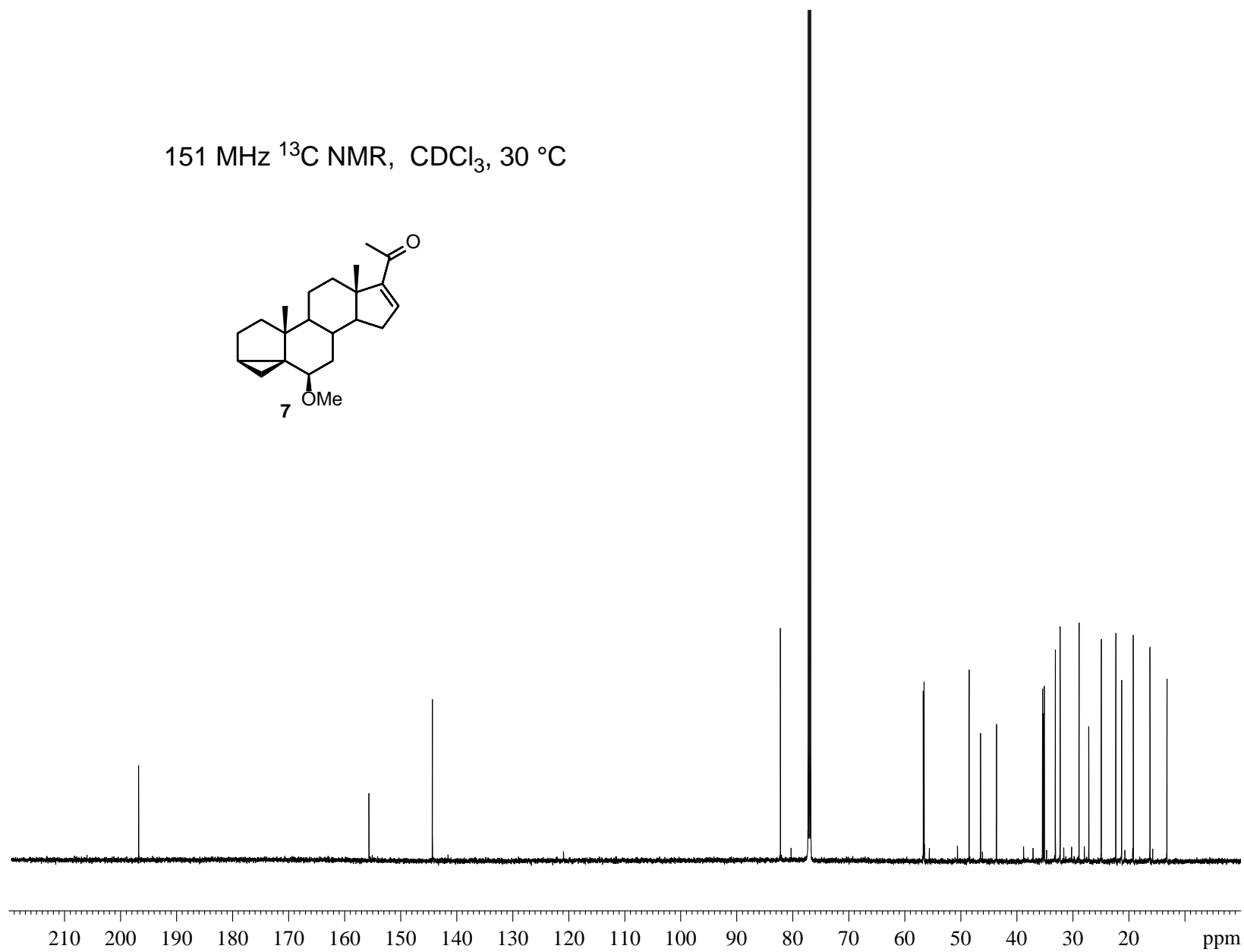
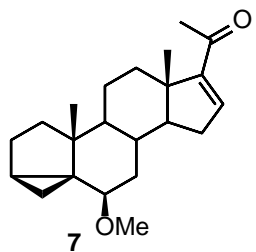
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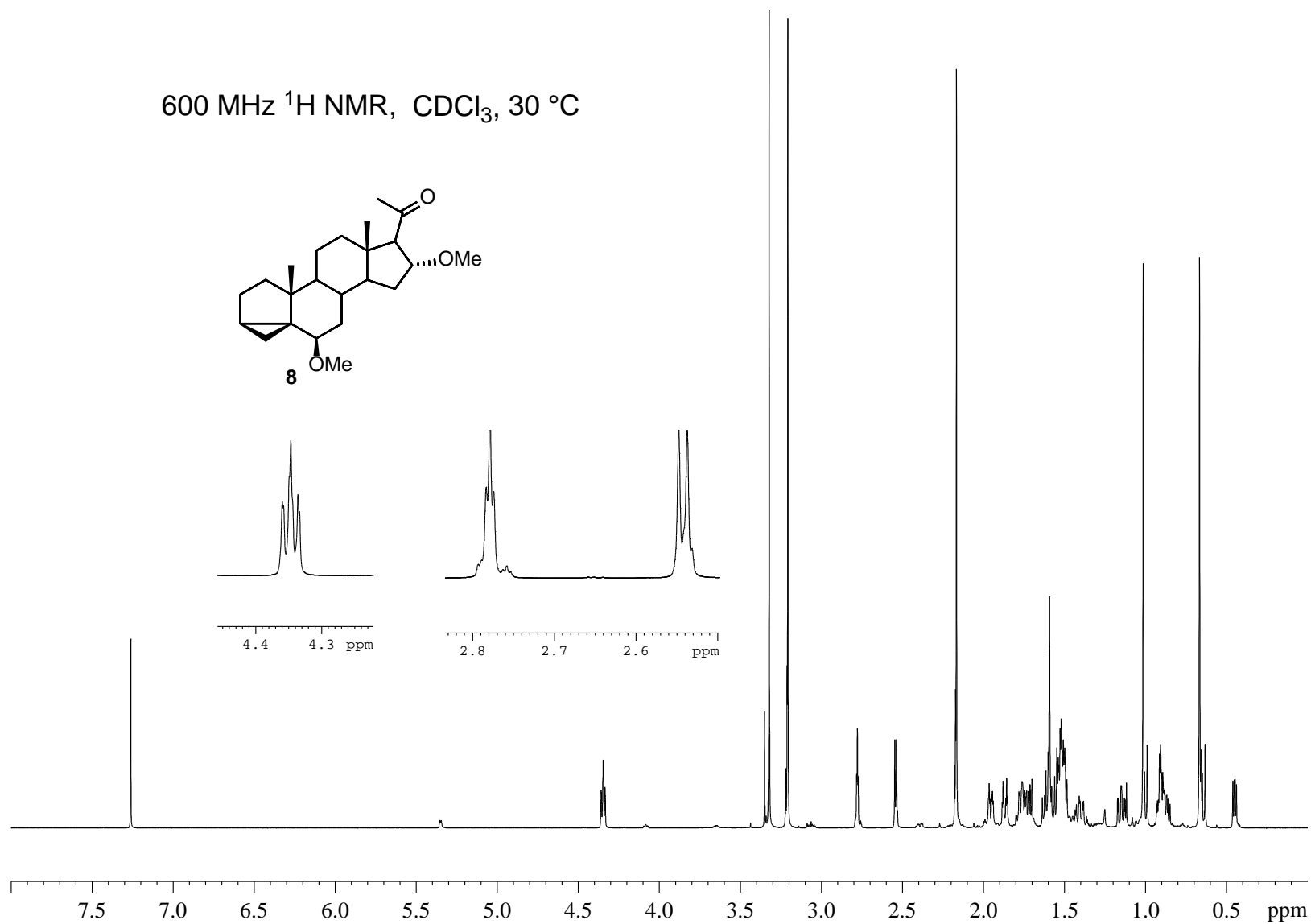
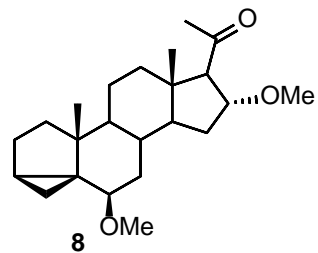
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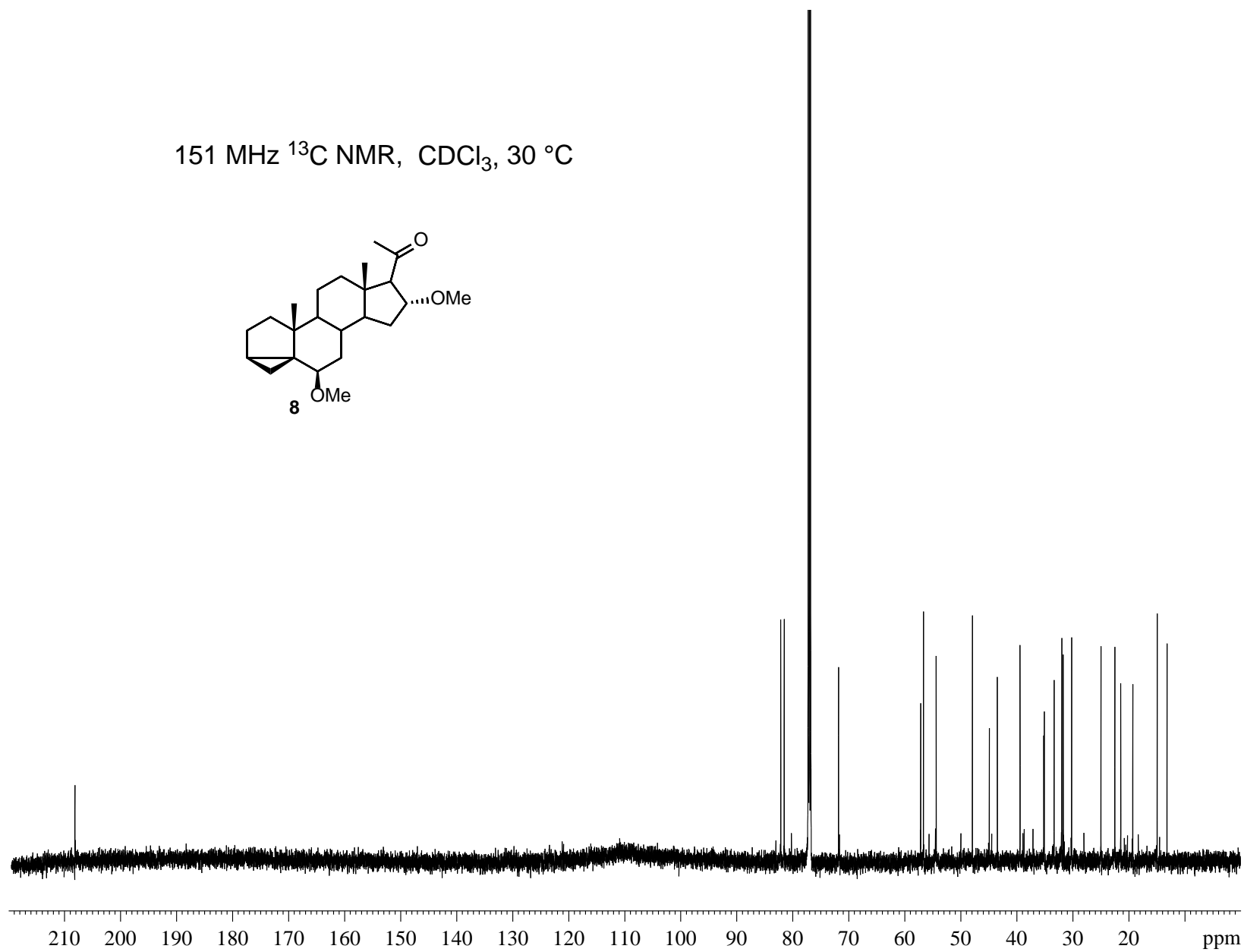
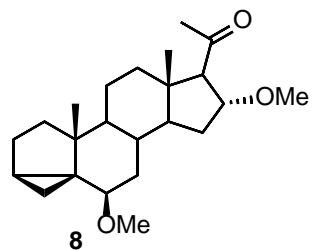
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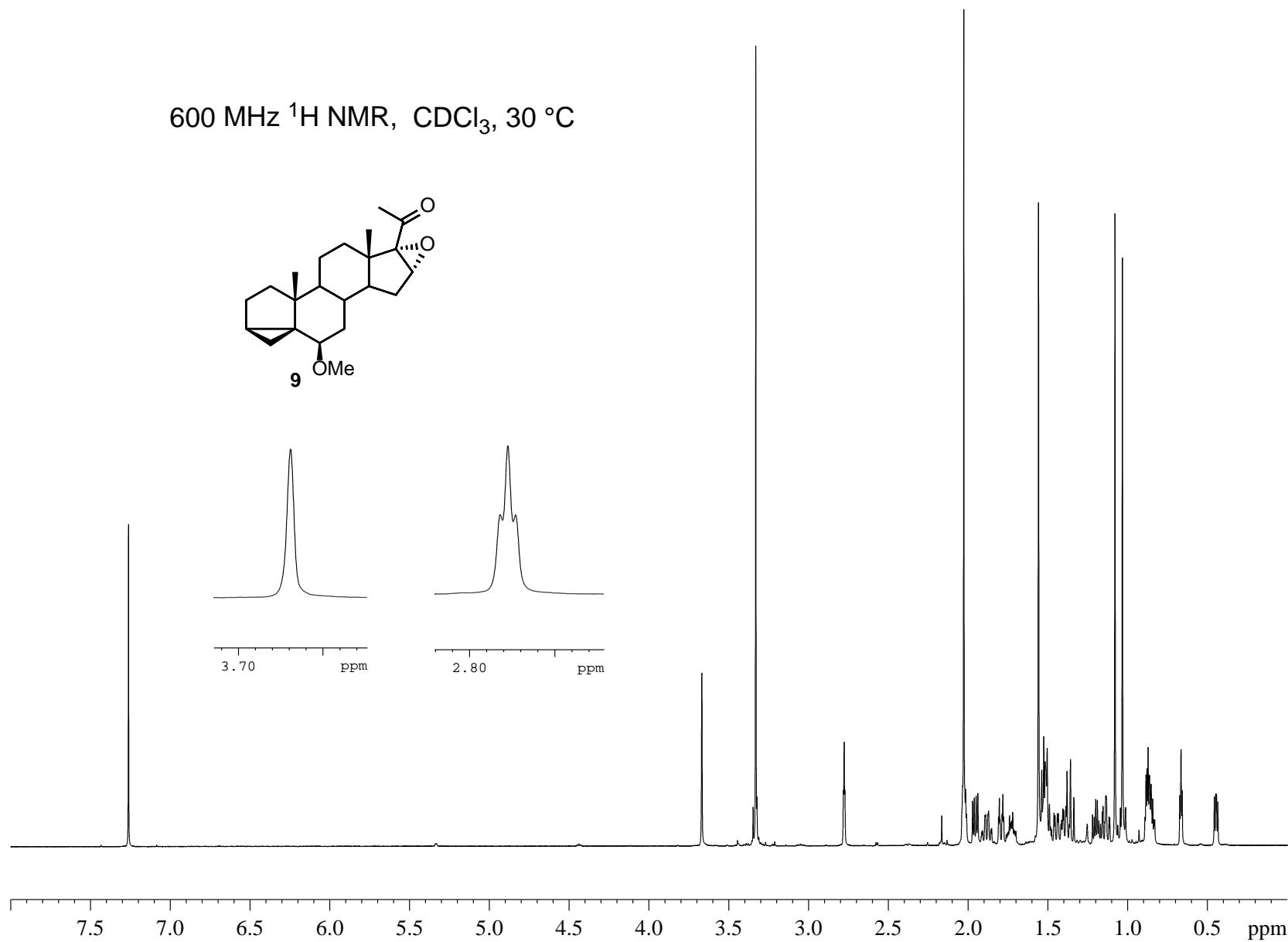
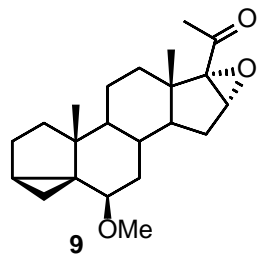
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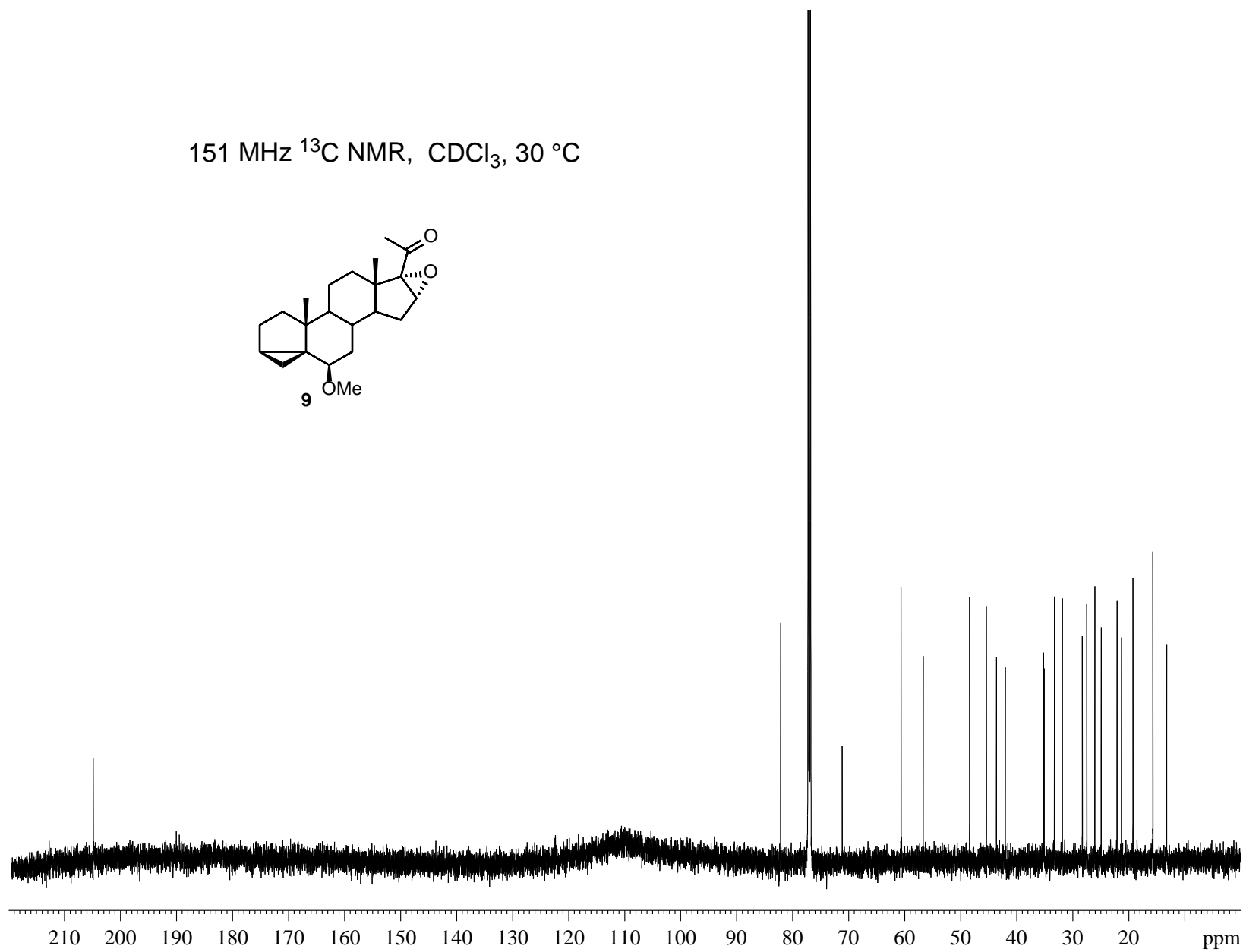
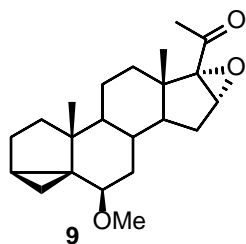
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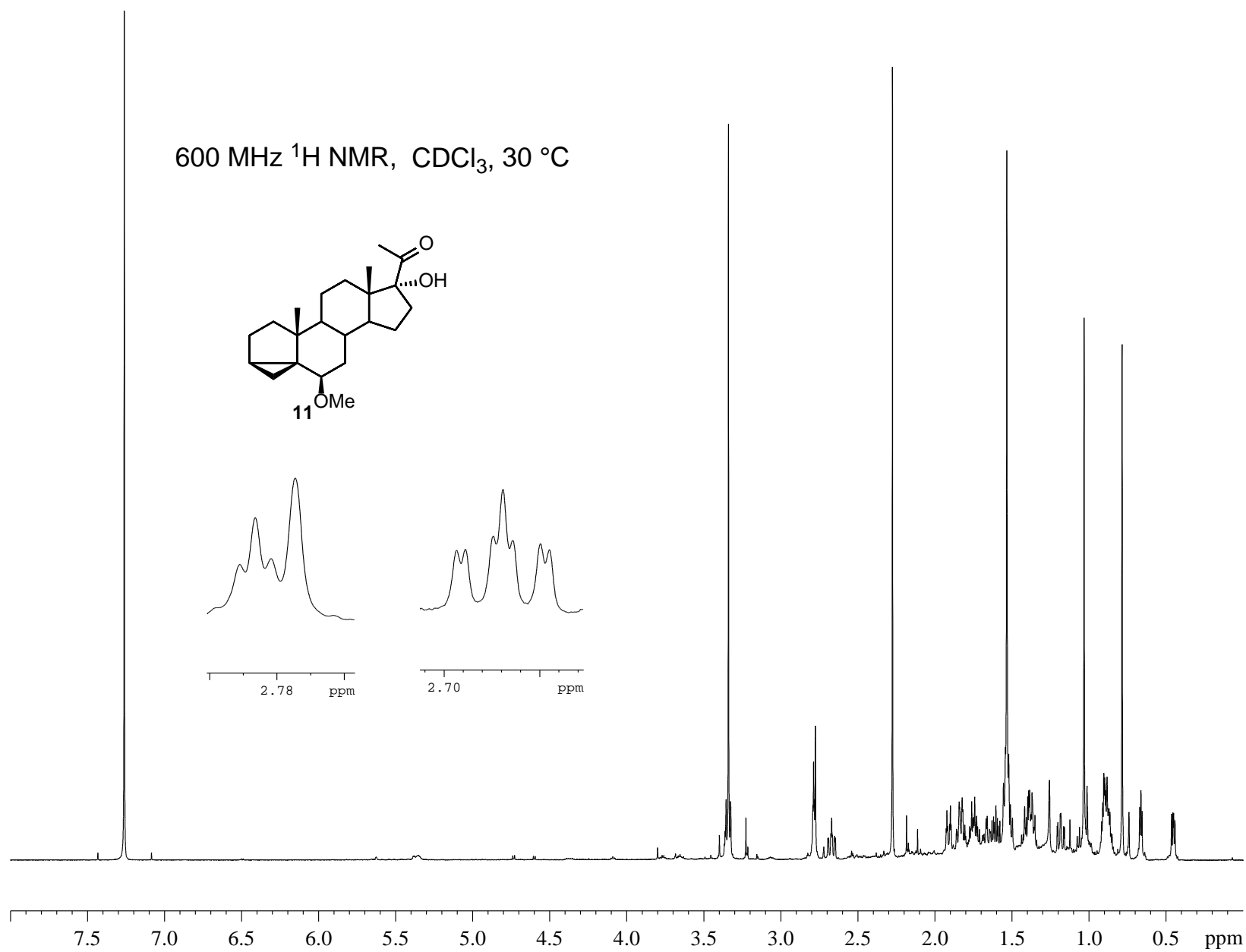
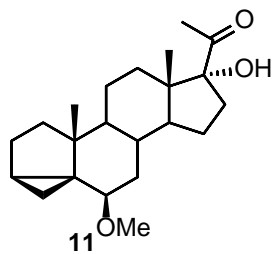
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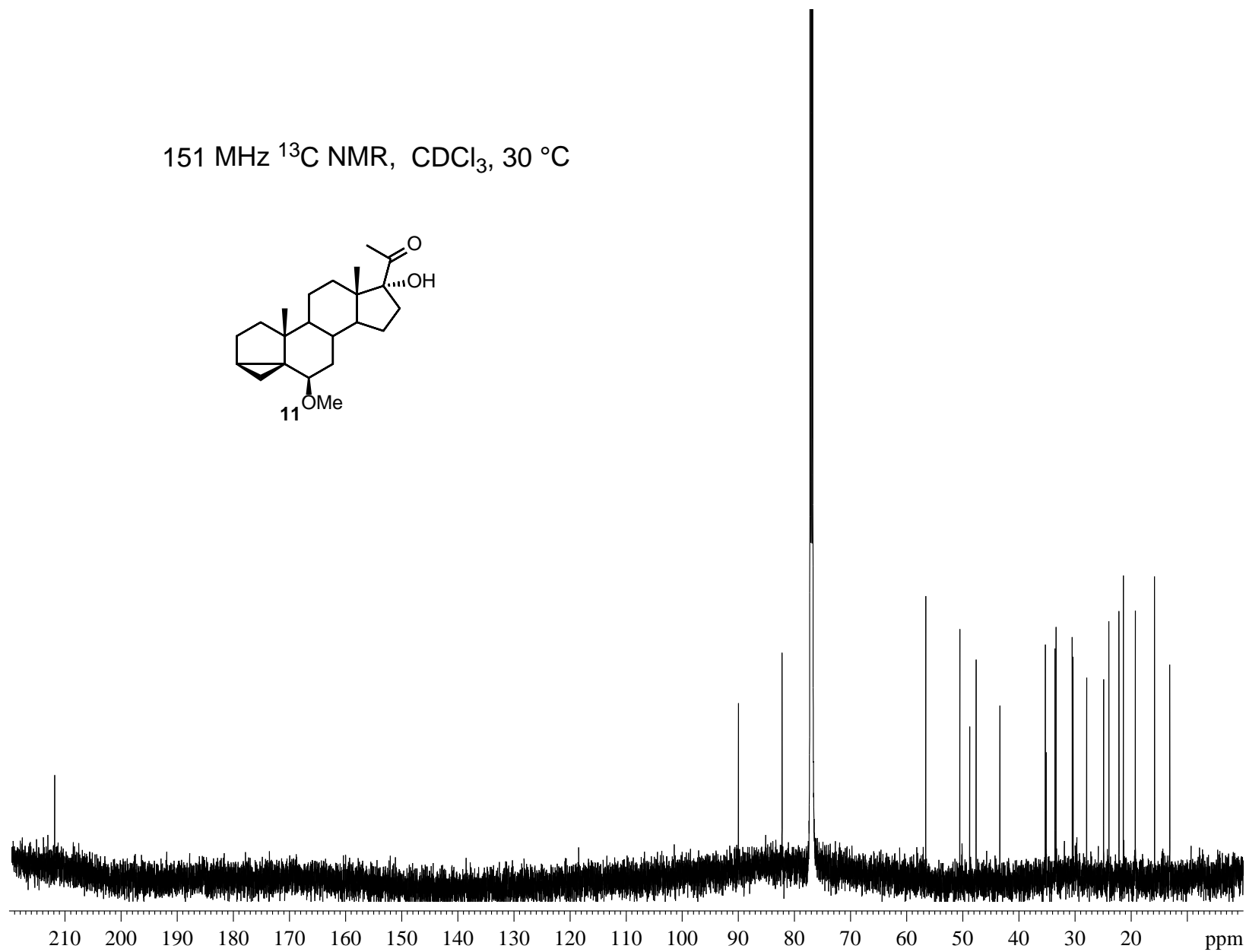
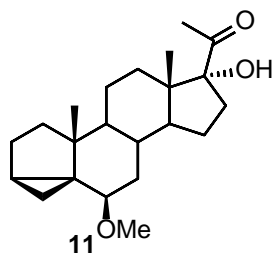
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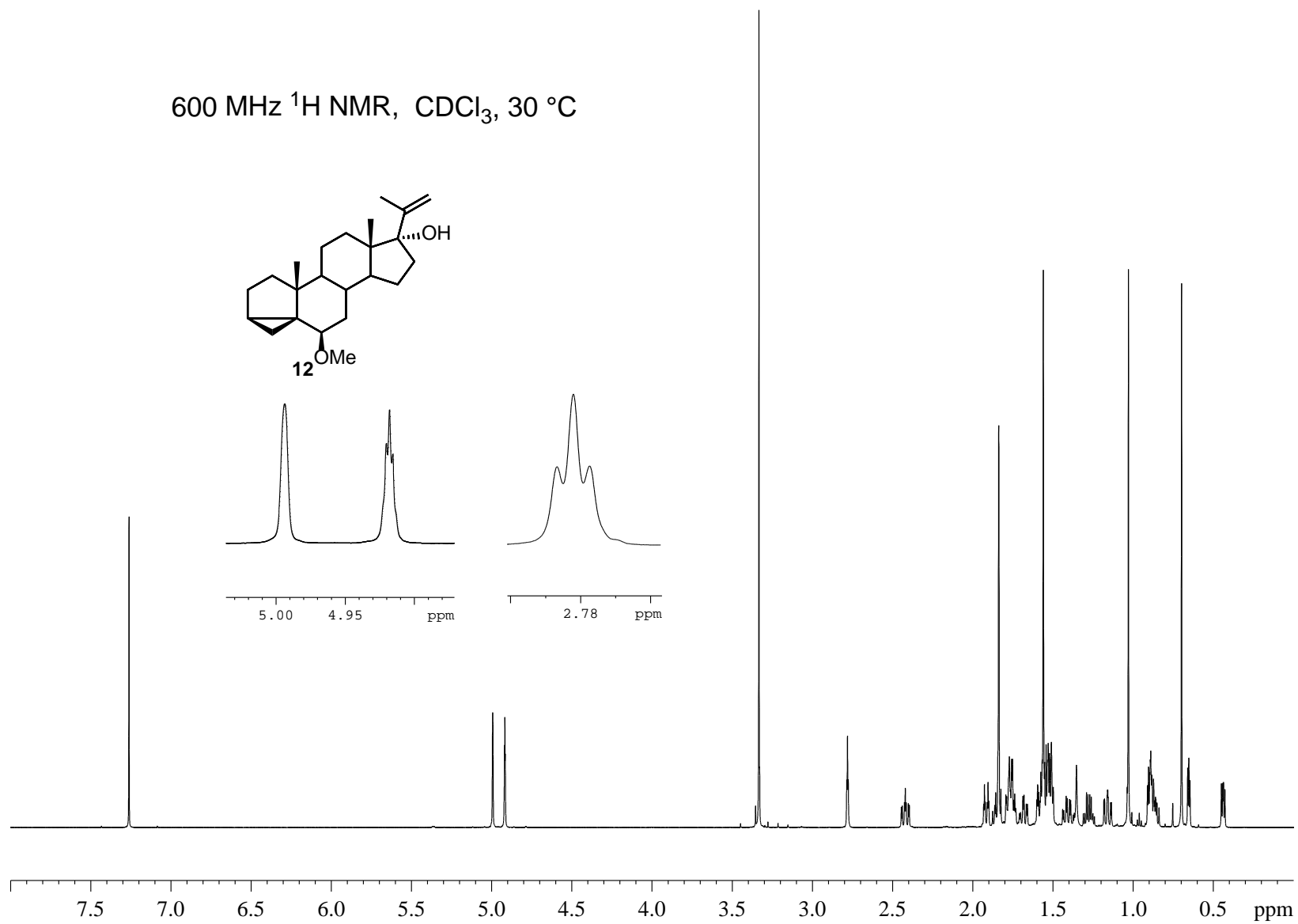
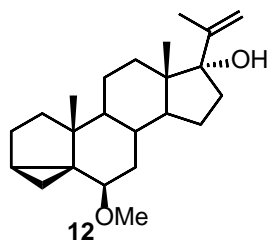
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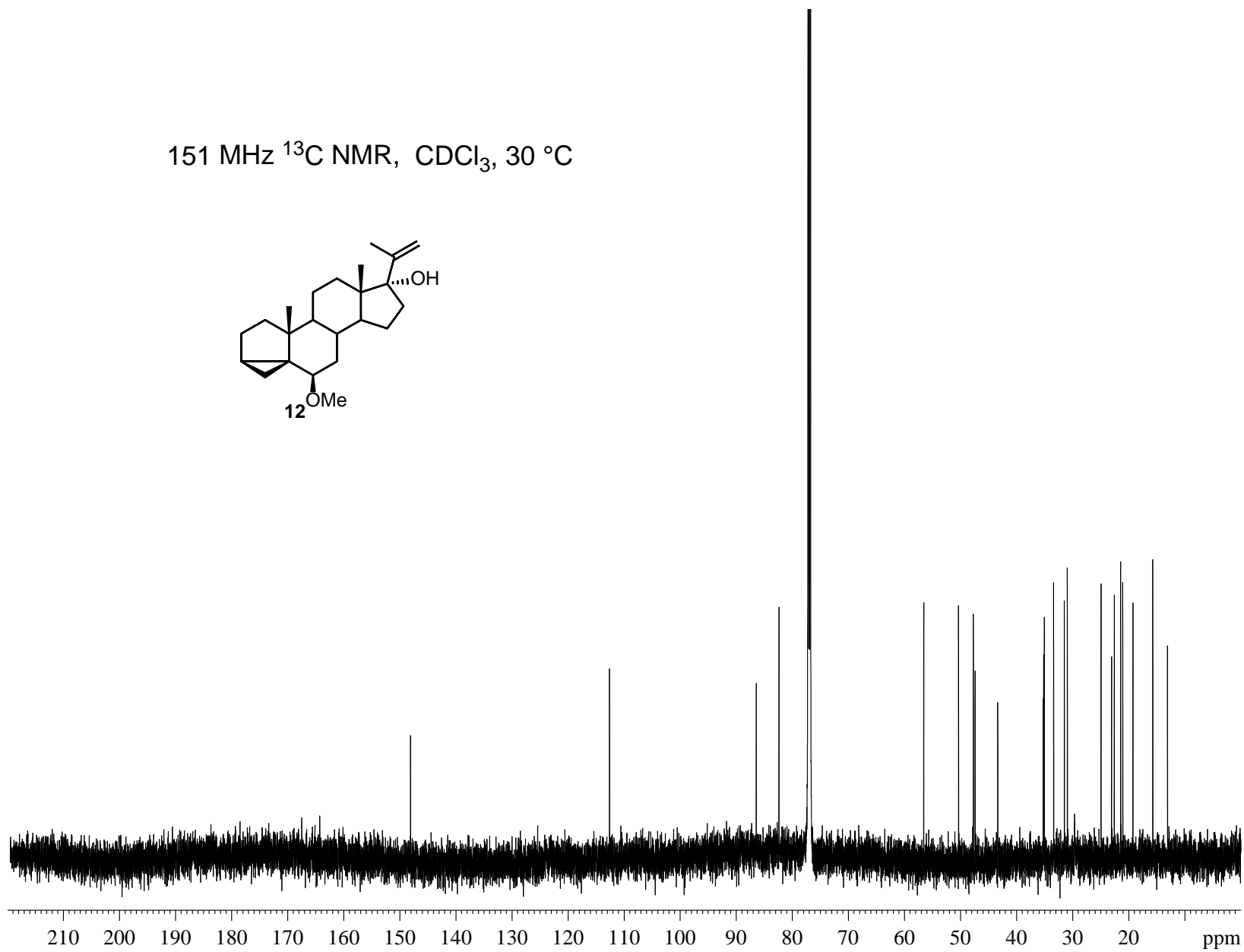
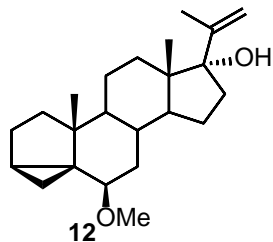
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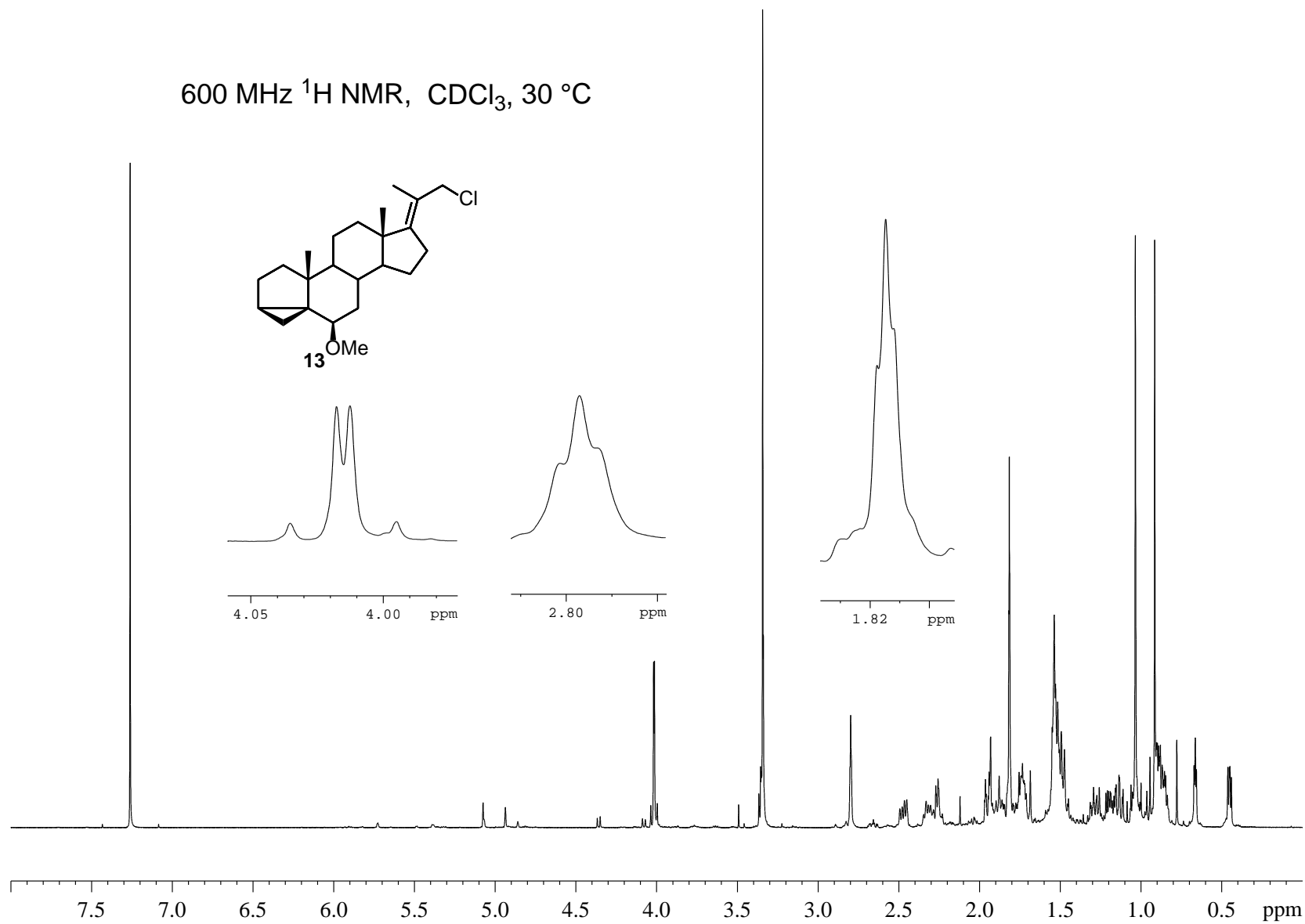
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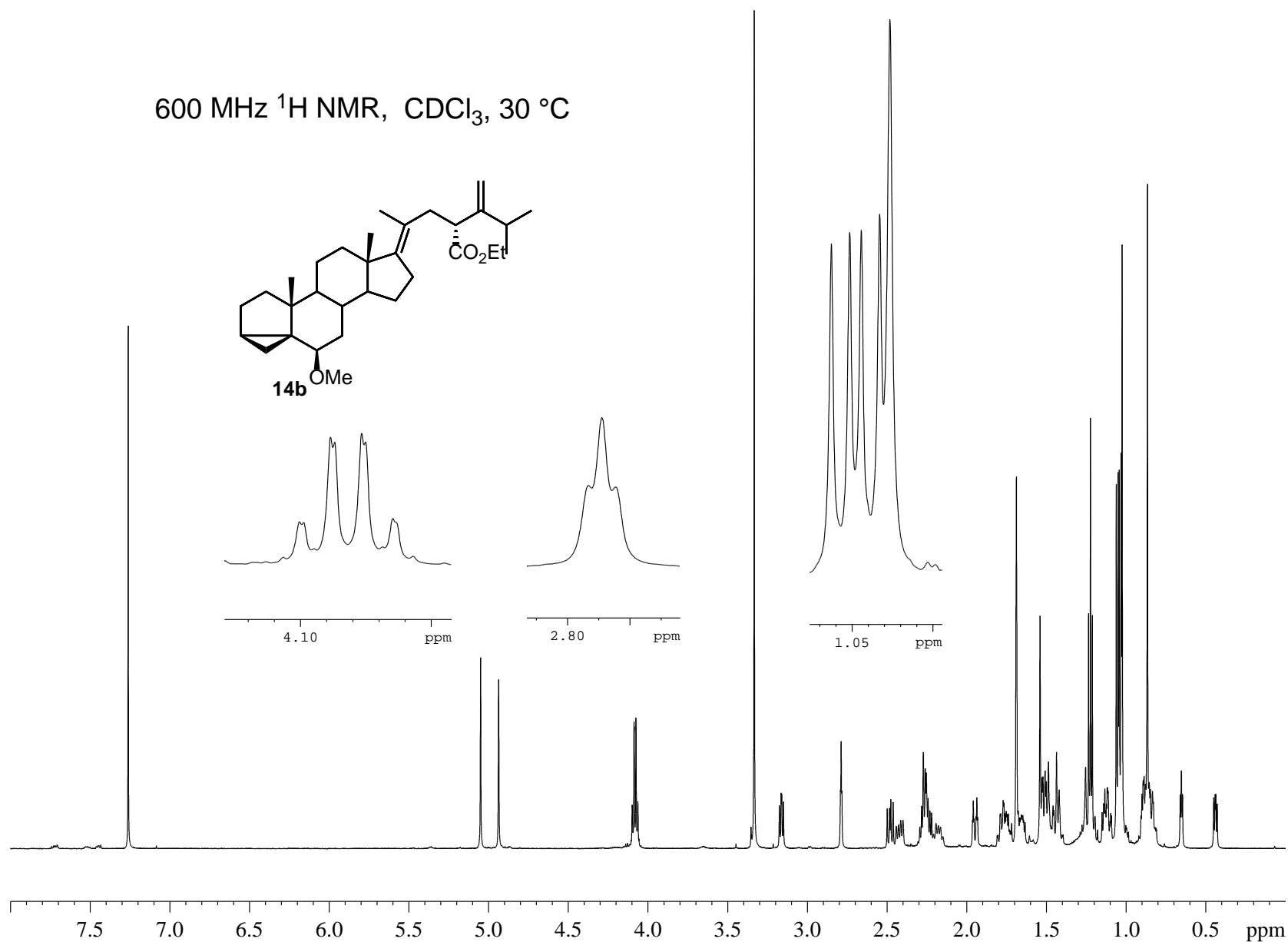
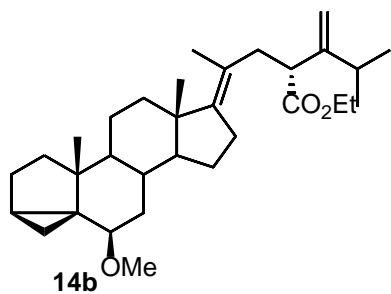
151 MHz ^{13}C NMR, CDCl_3 , 30 °C



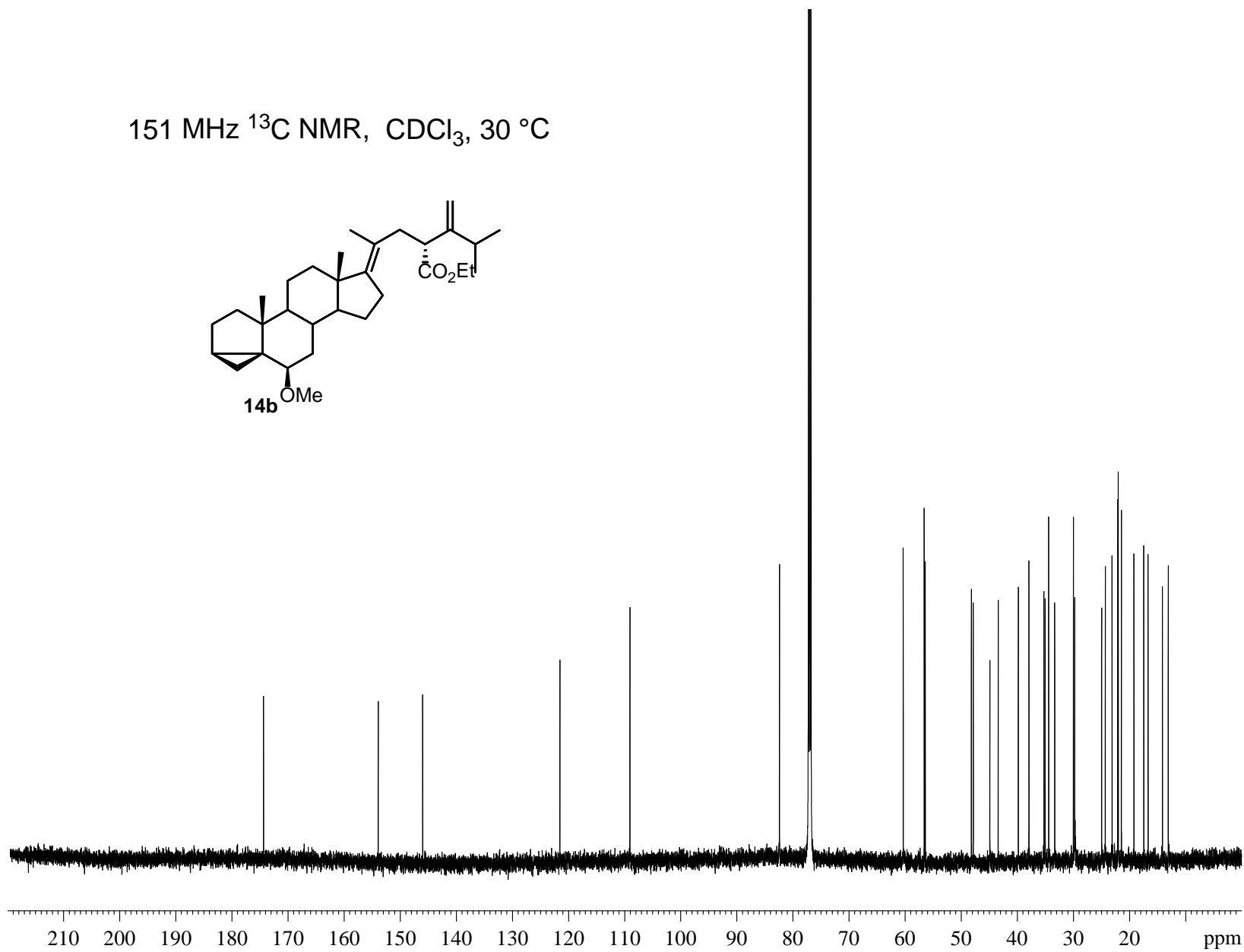
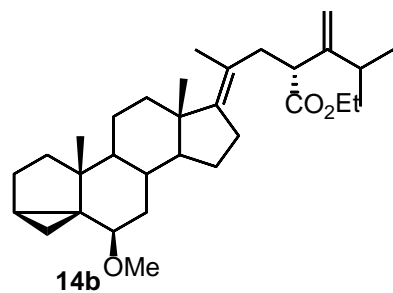
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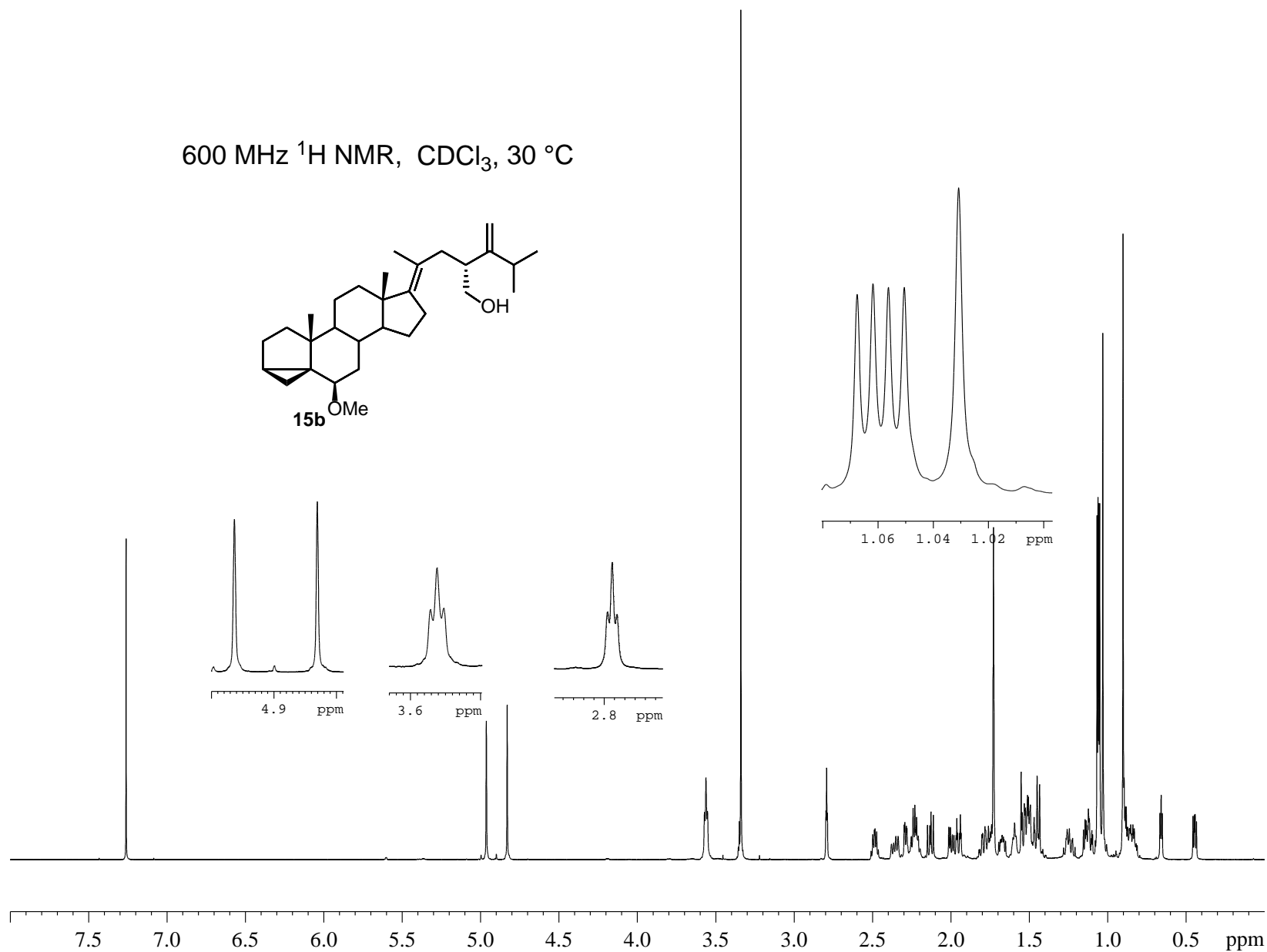
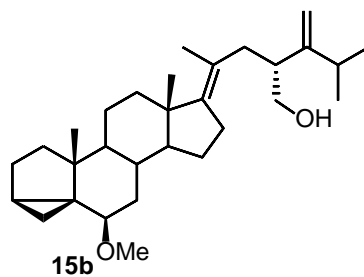
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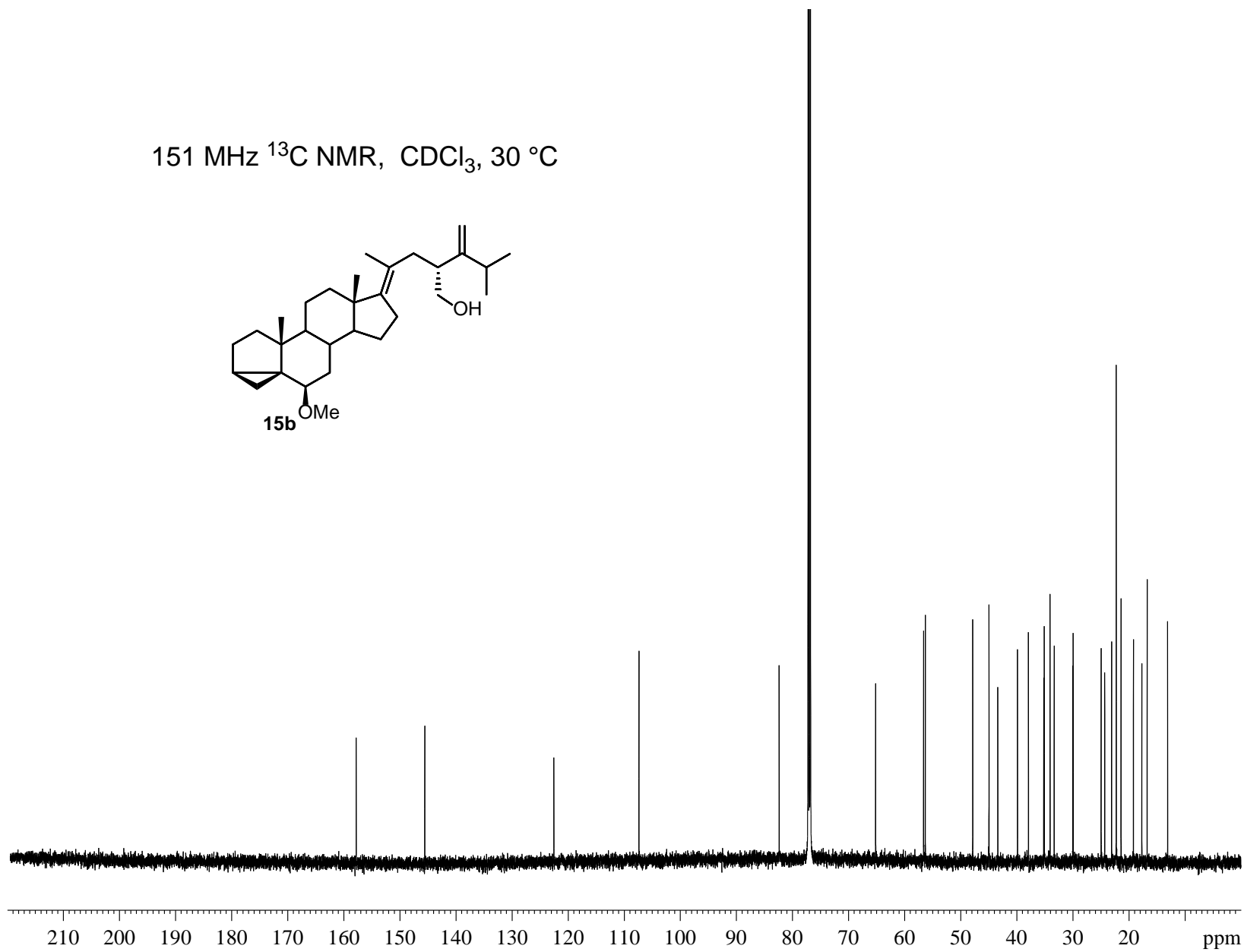
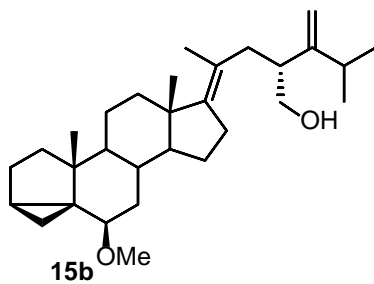
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



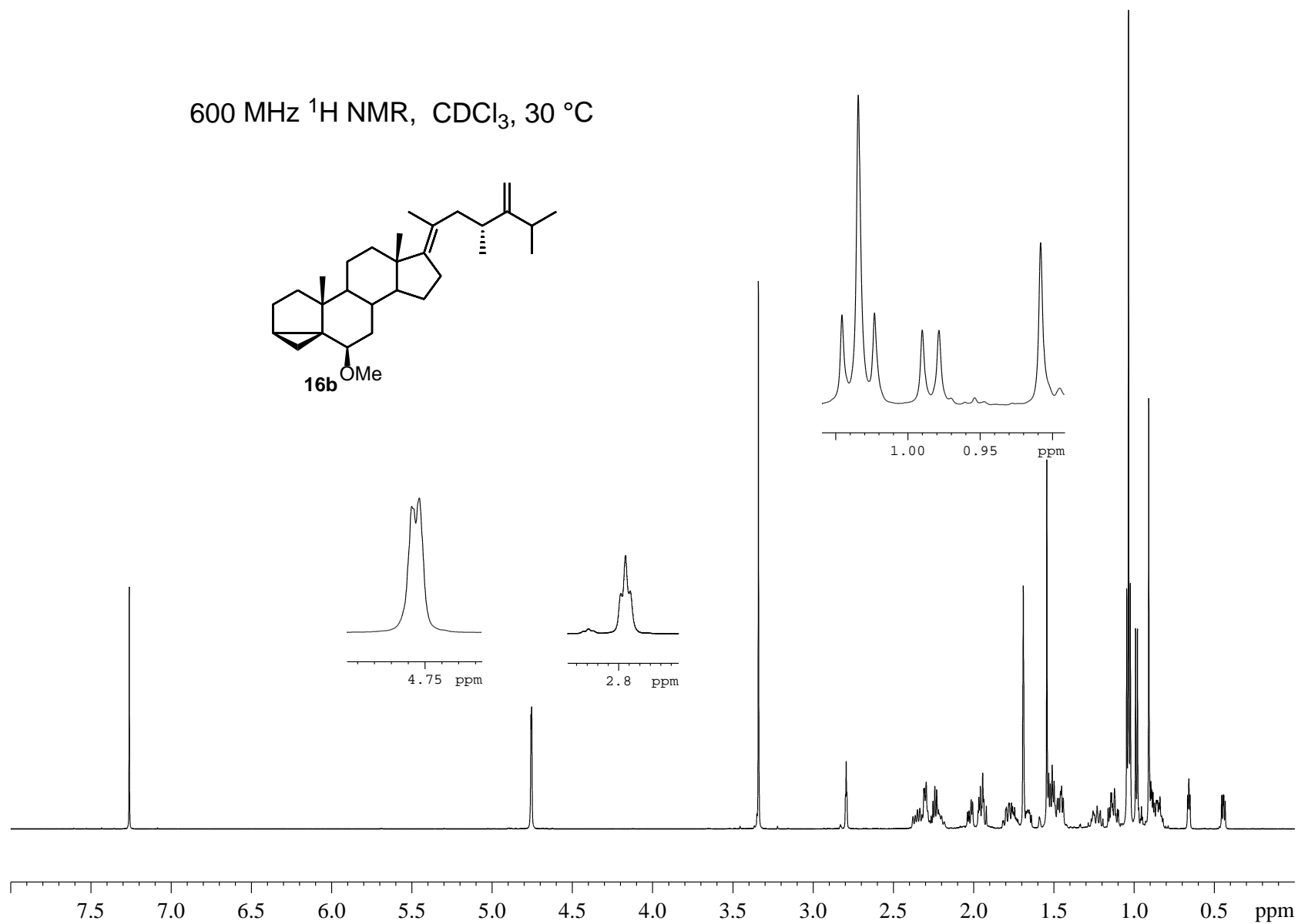
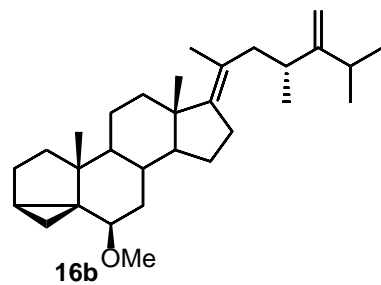
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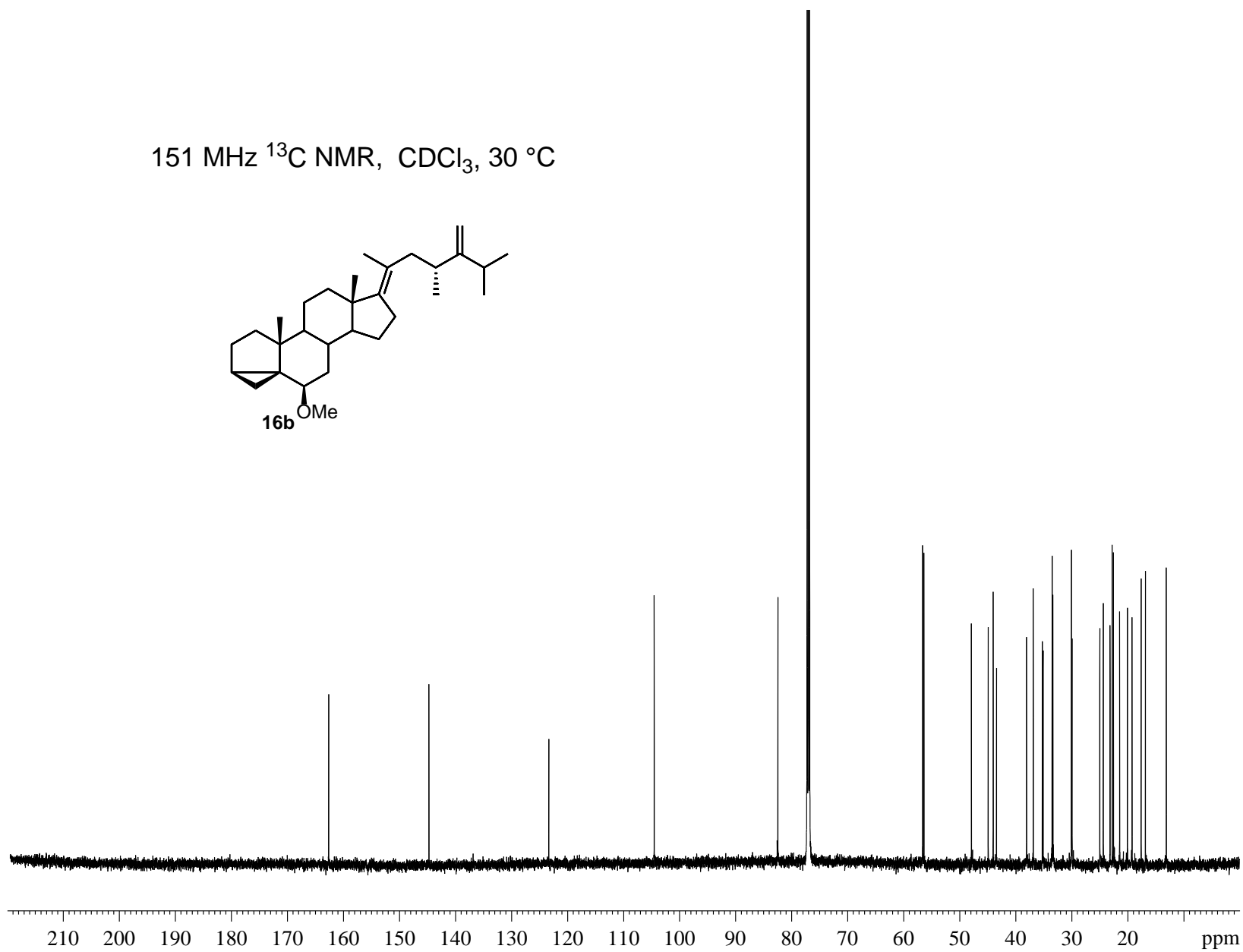
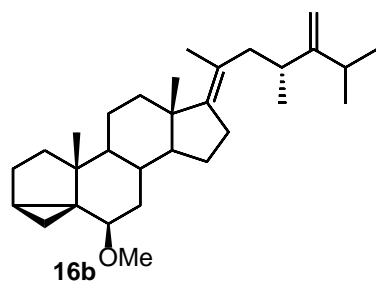
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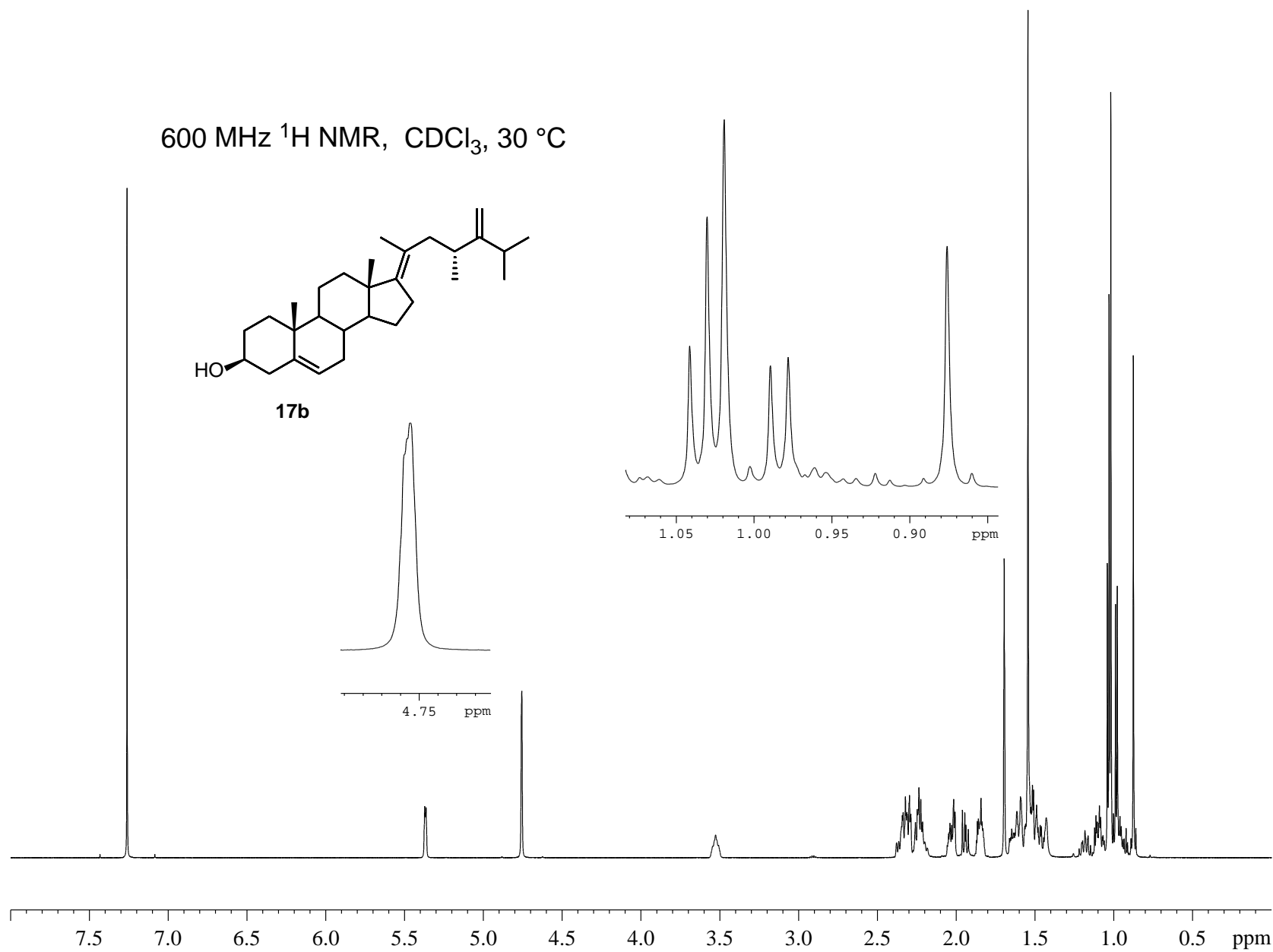
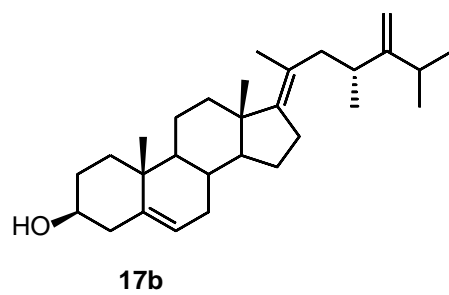
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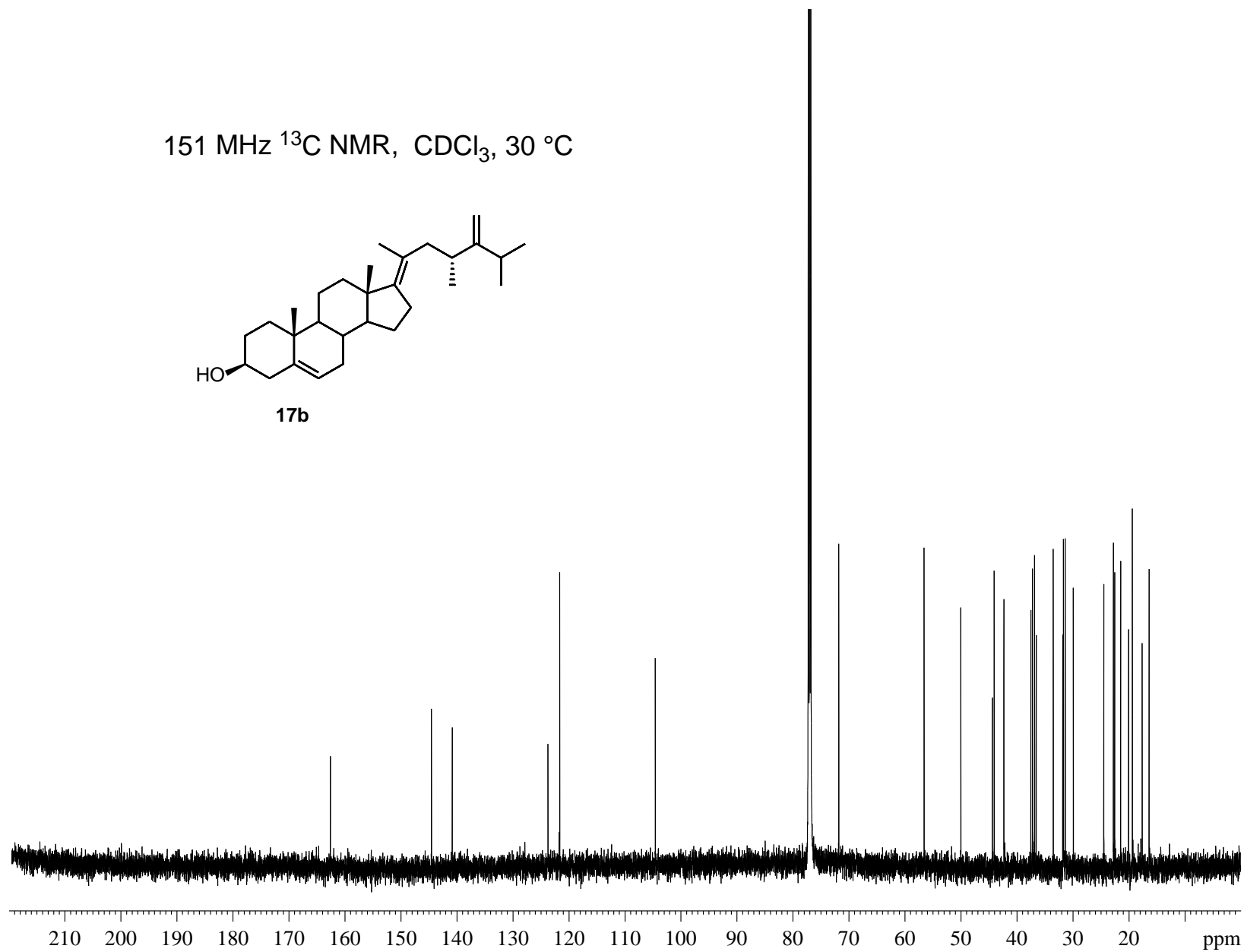
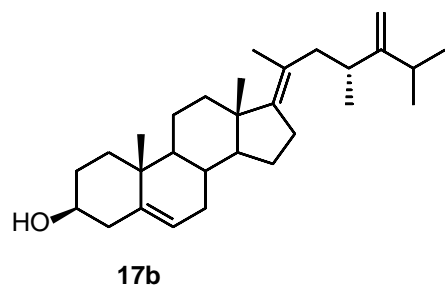
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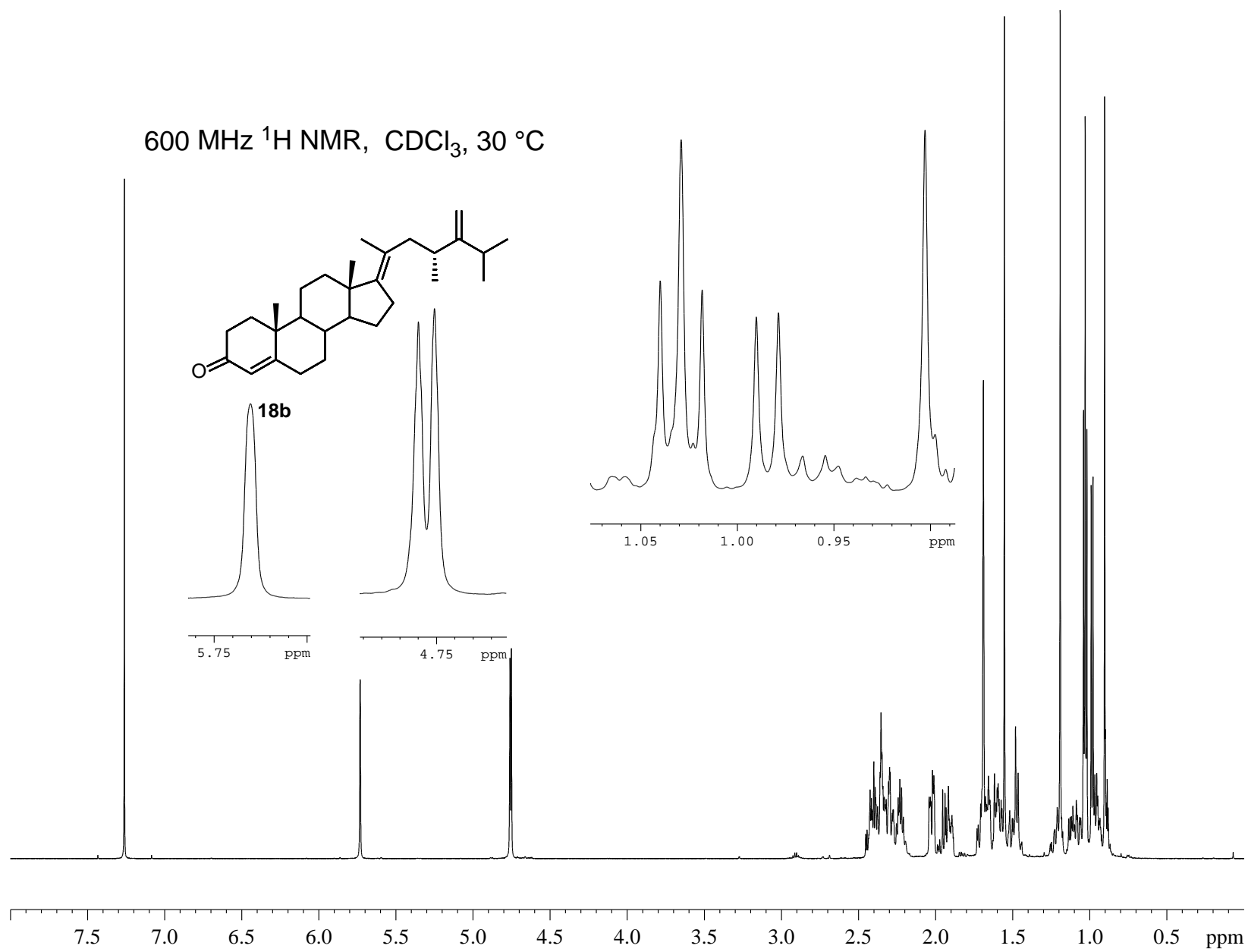
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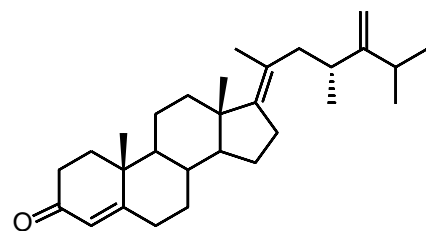
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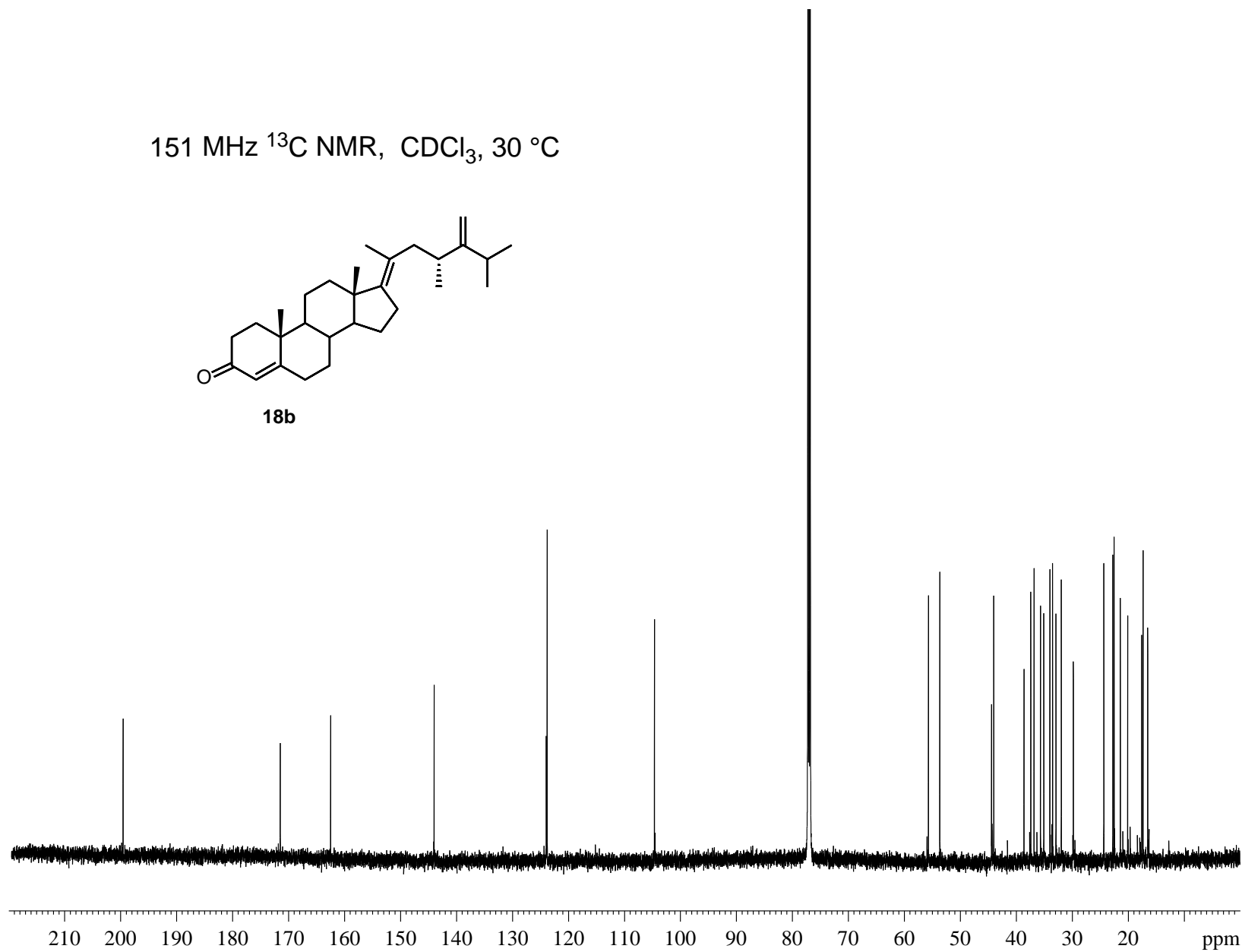
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



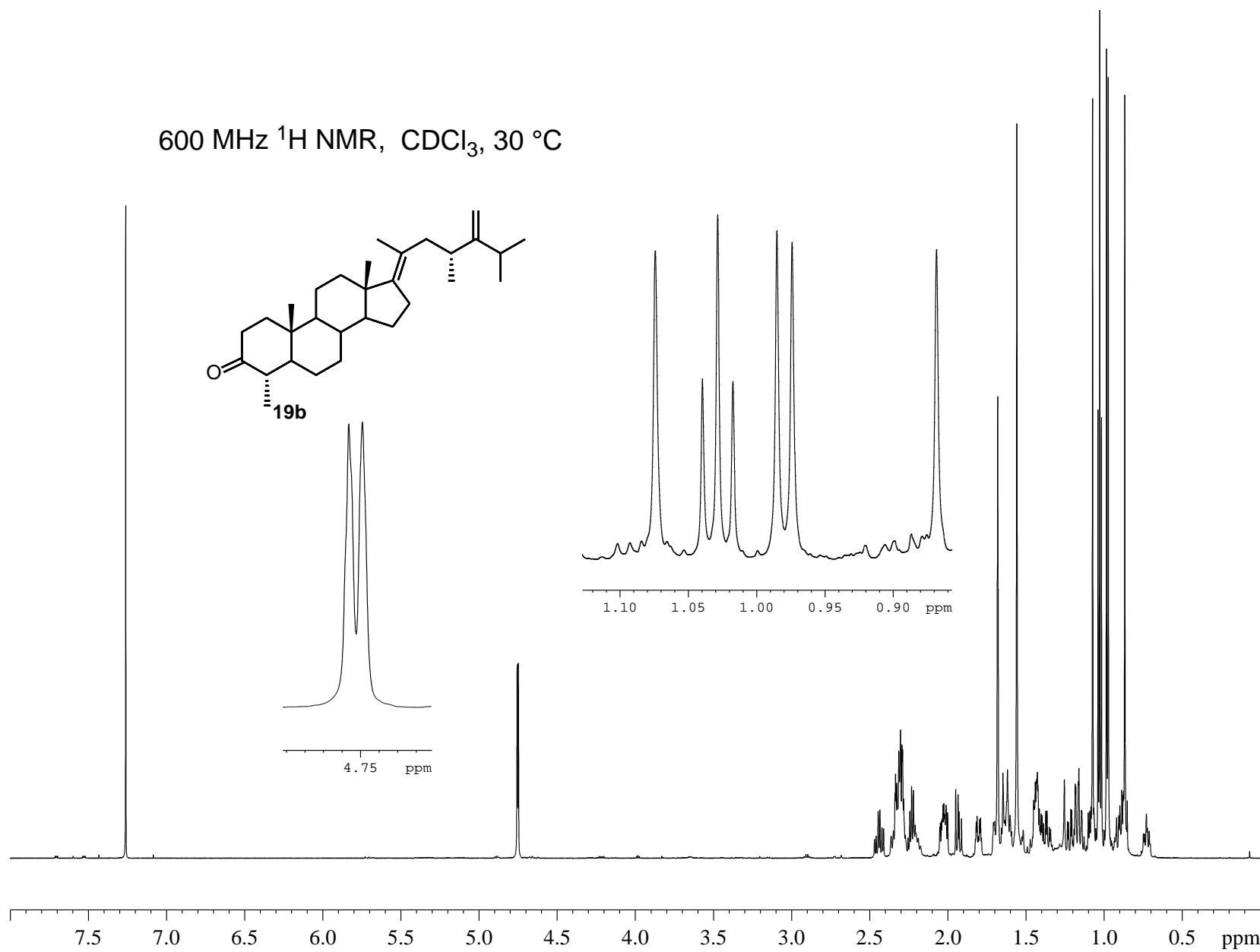
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



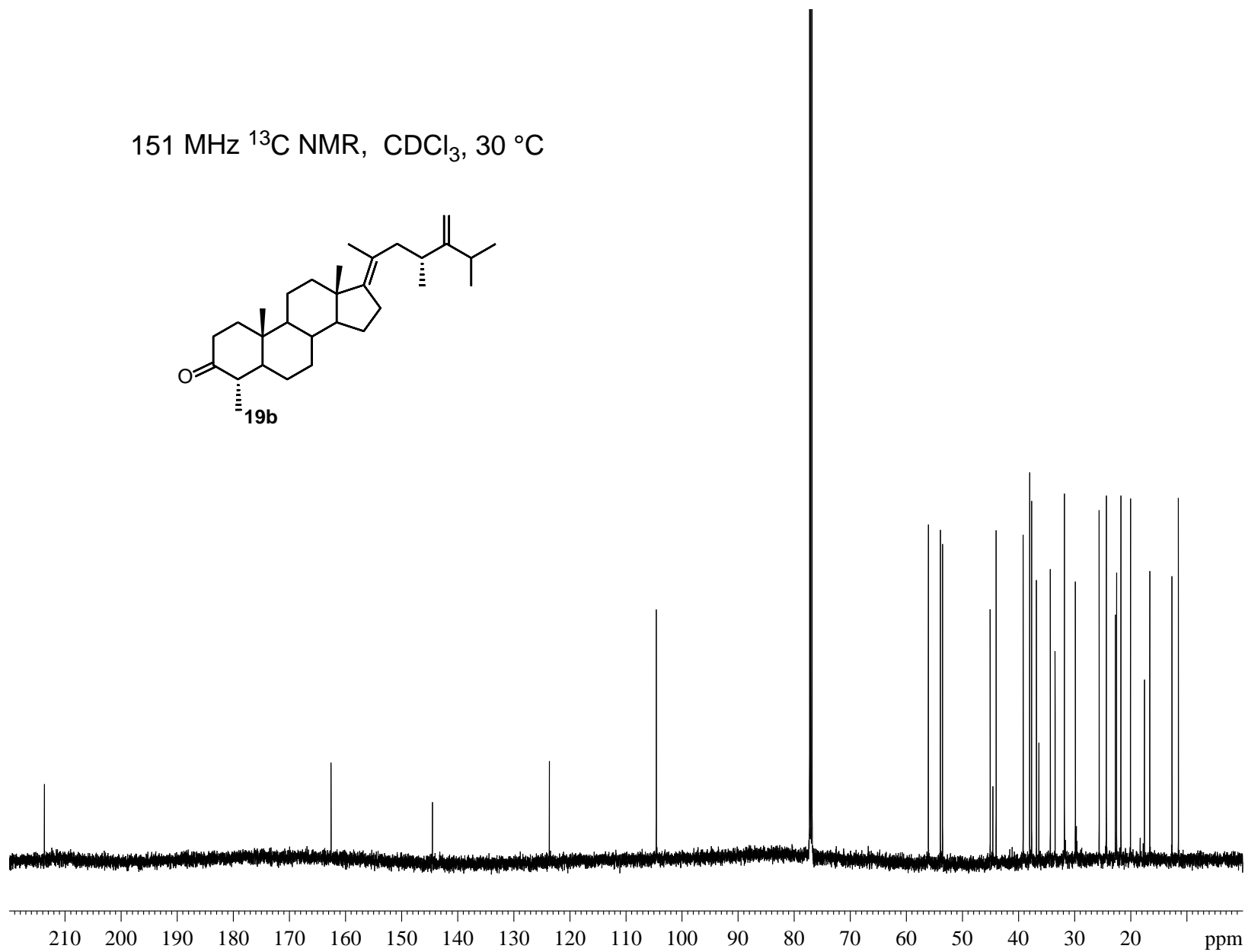
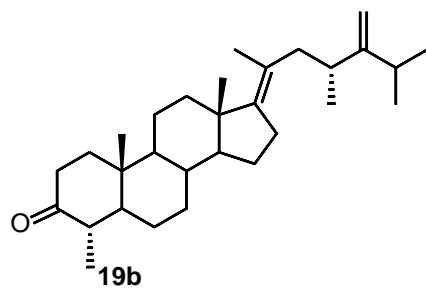
18b



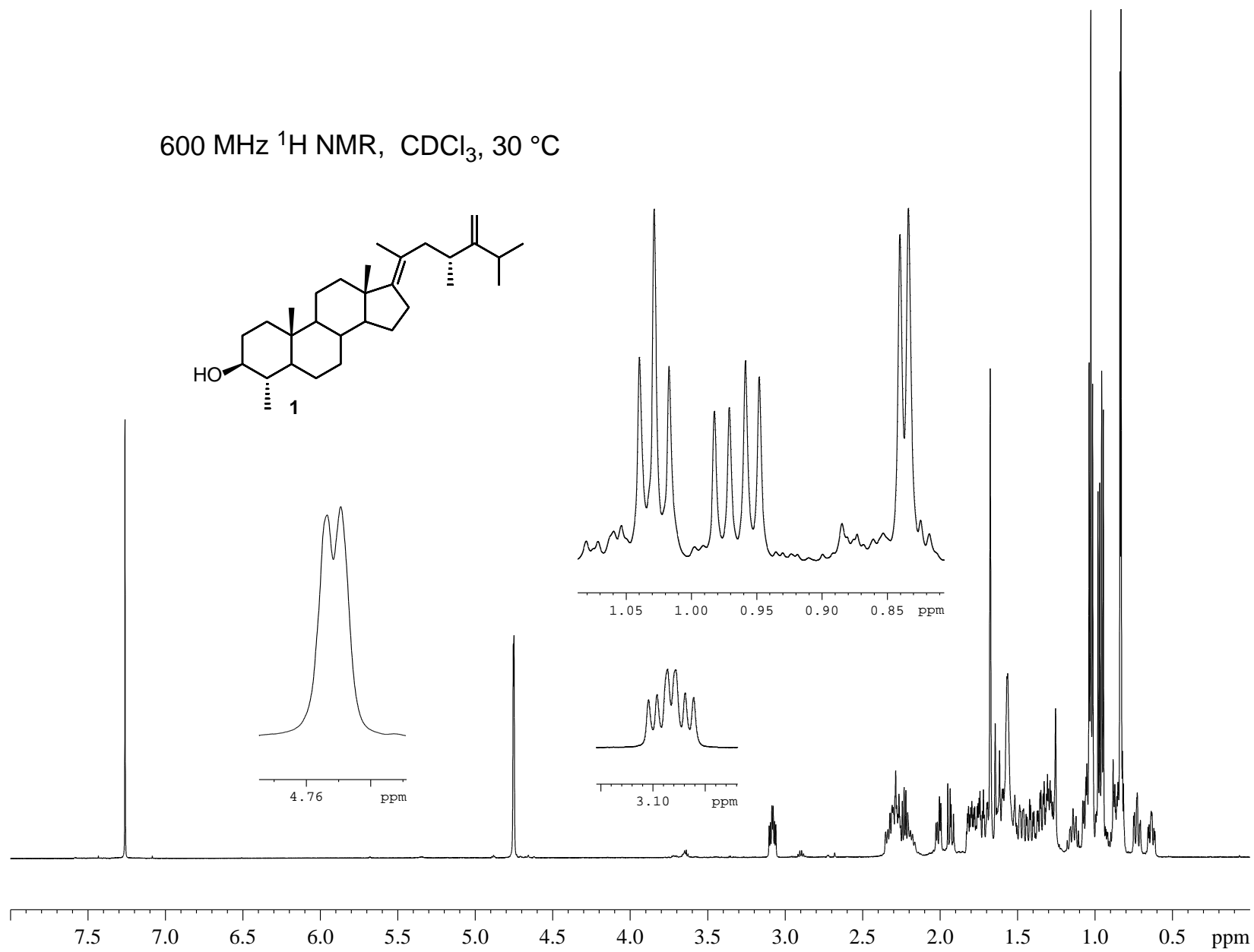
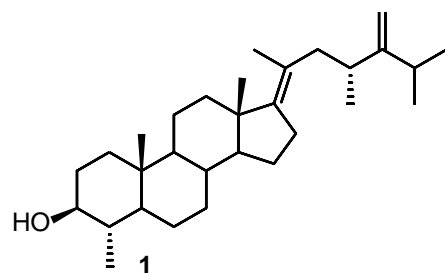
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



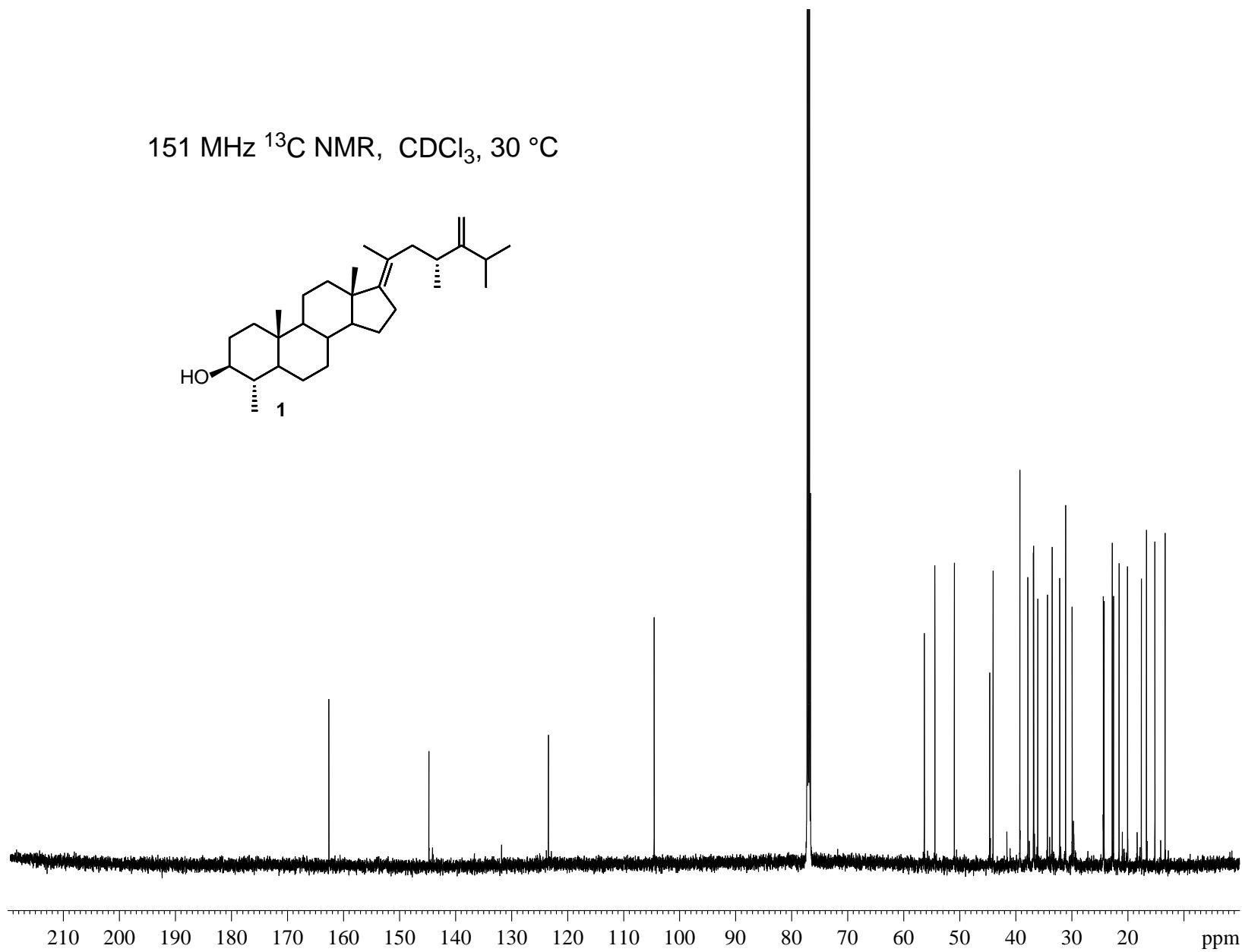
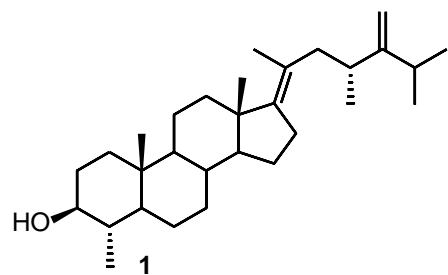
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



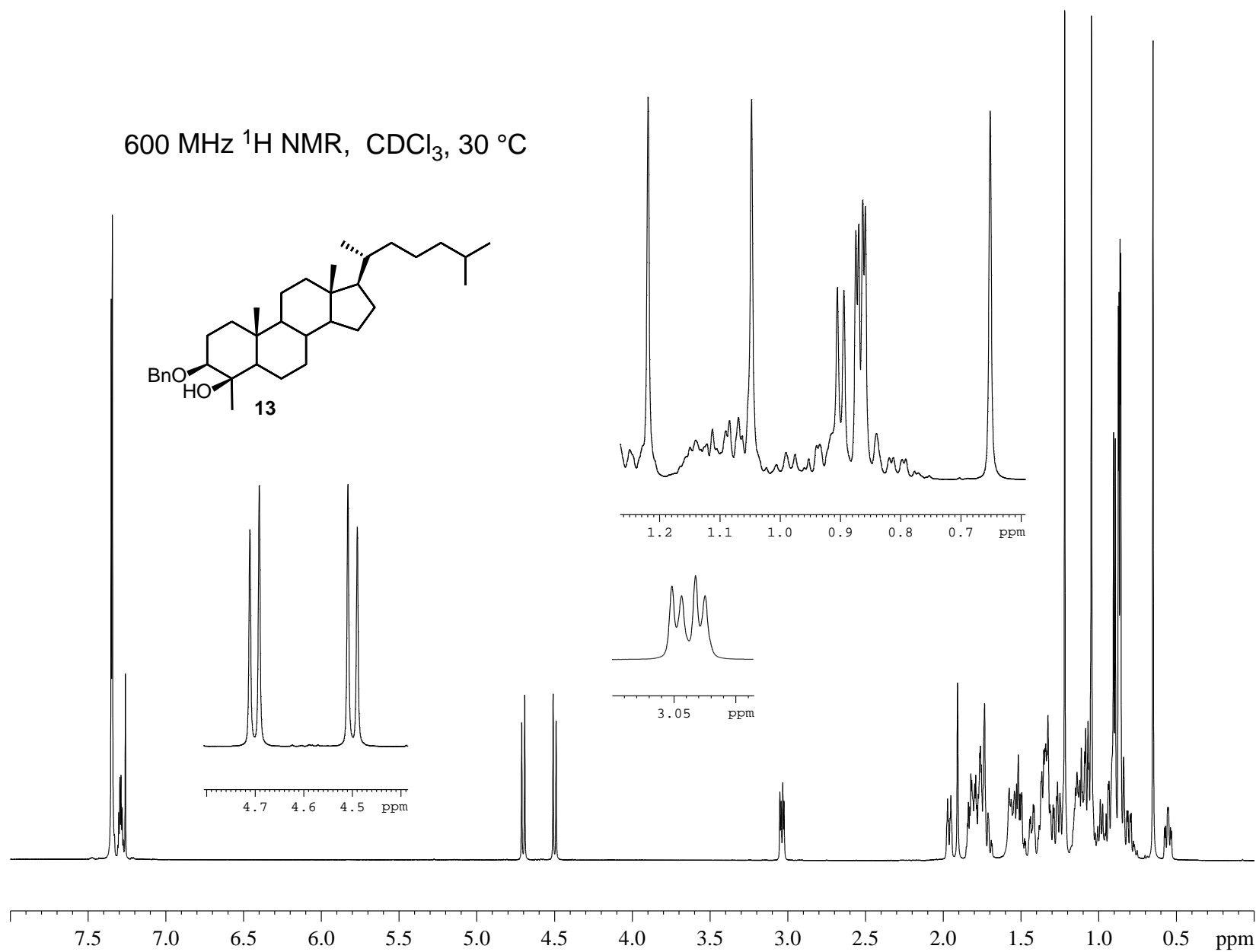
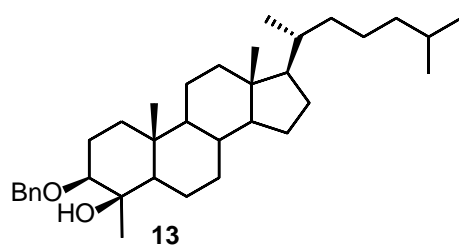
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



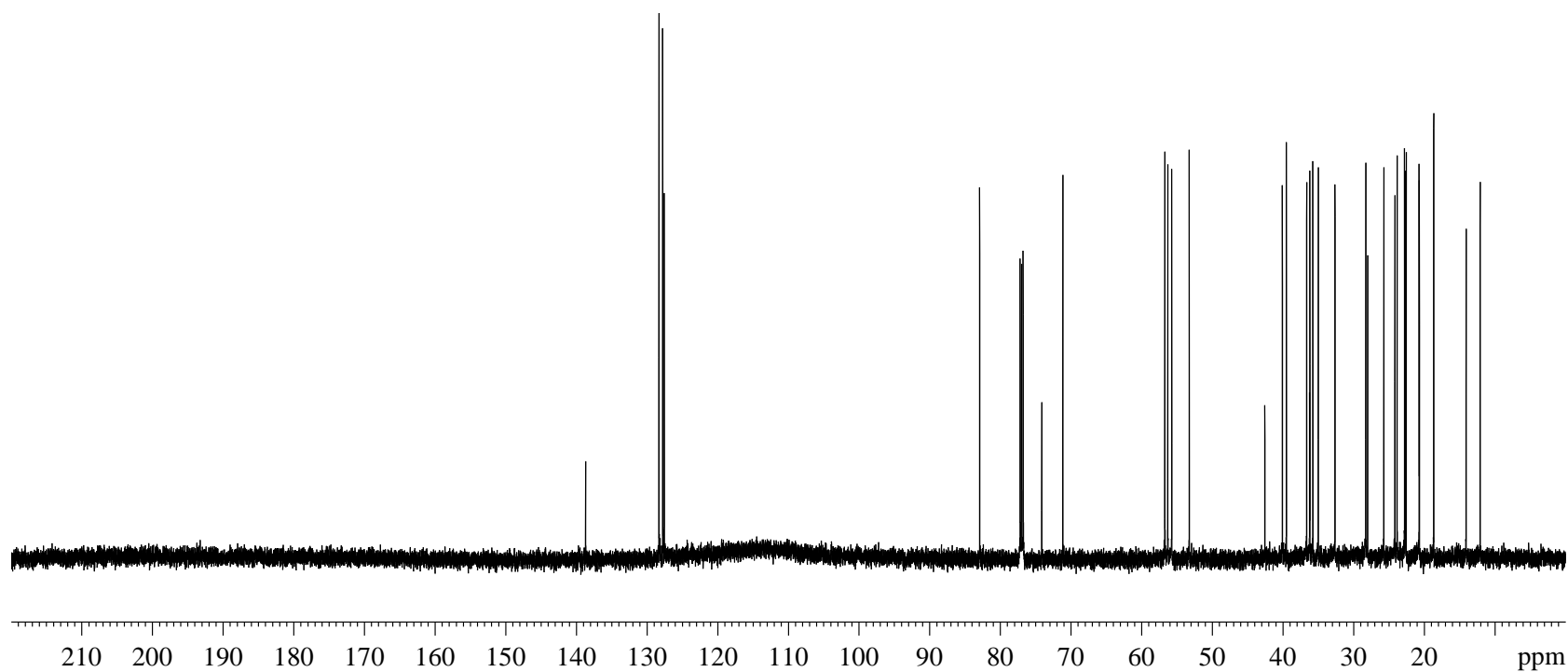
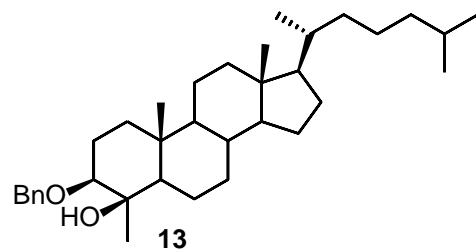
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



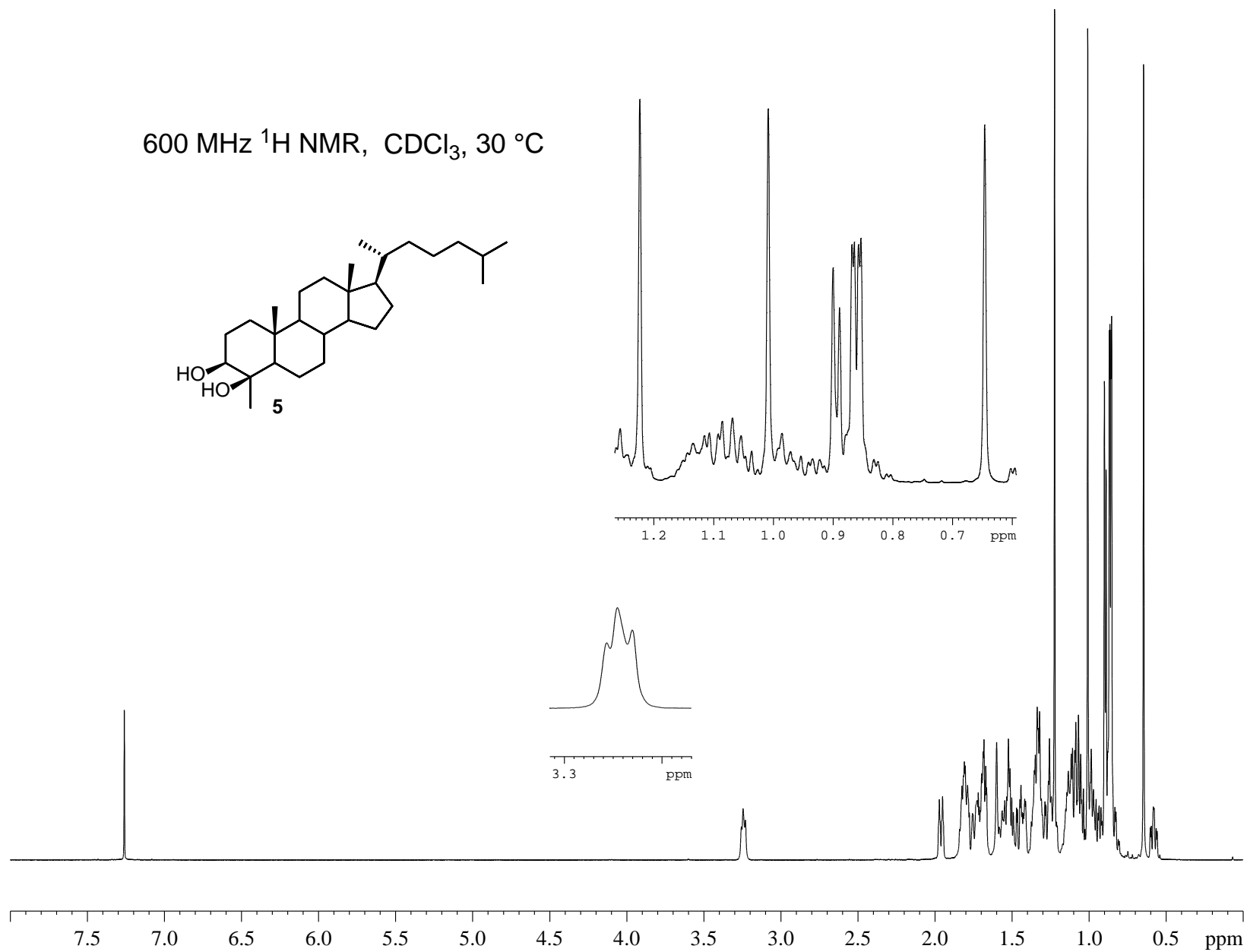
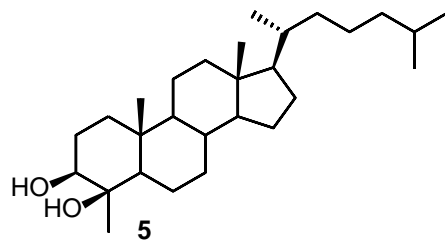
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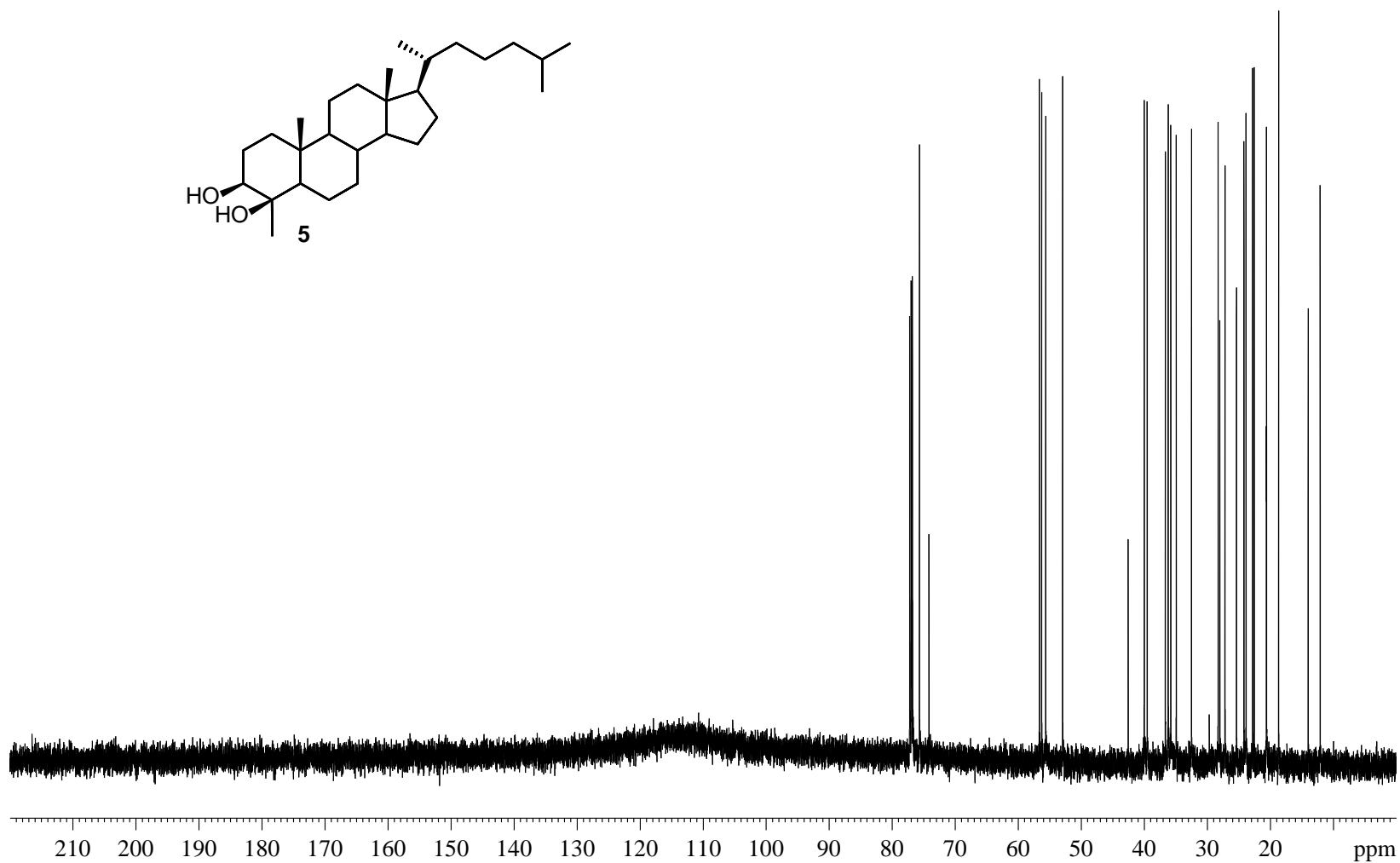
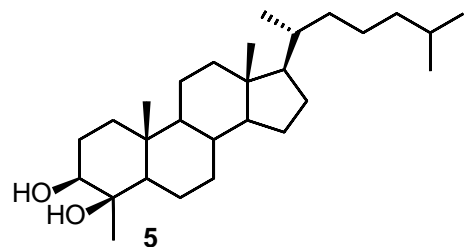
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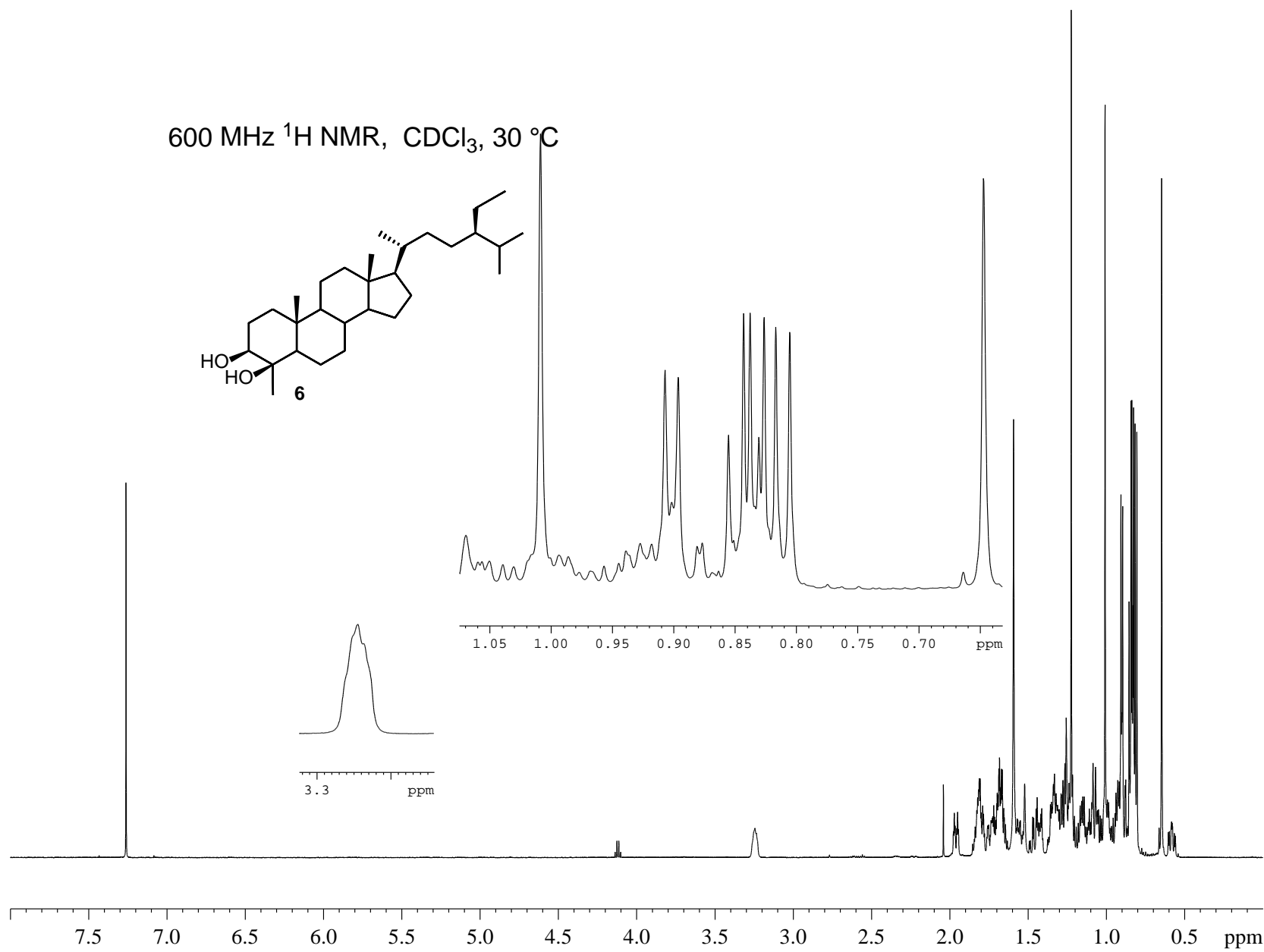
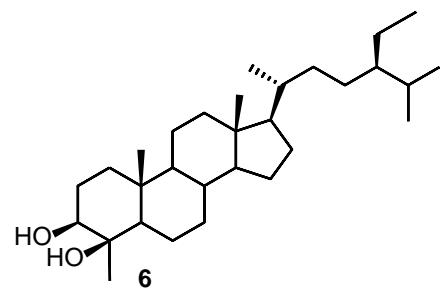
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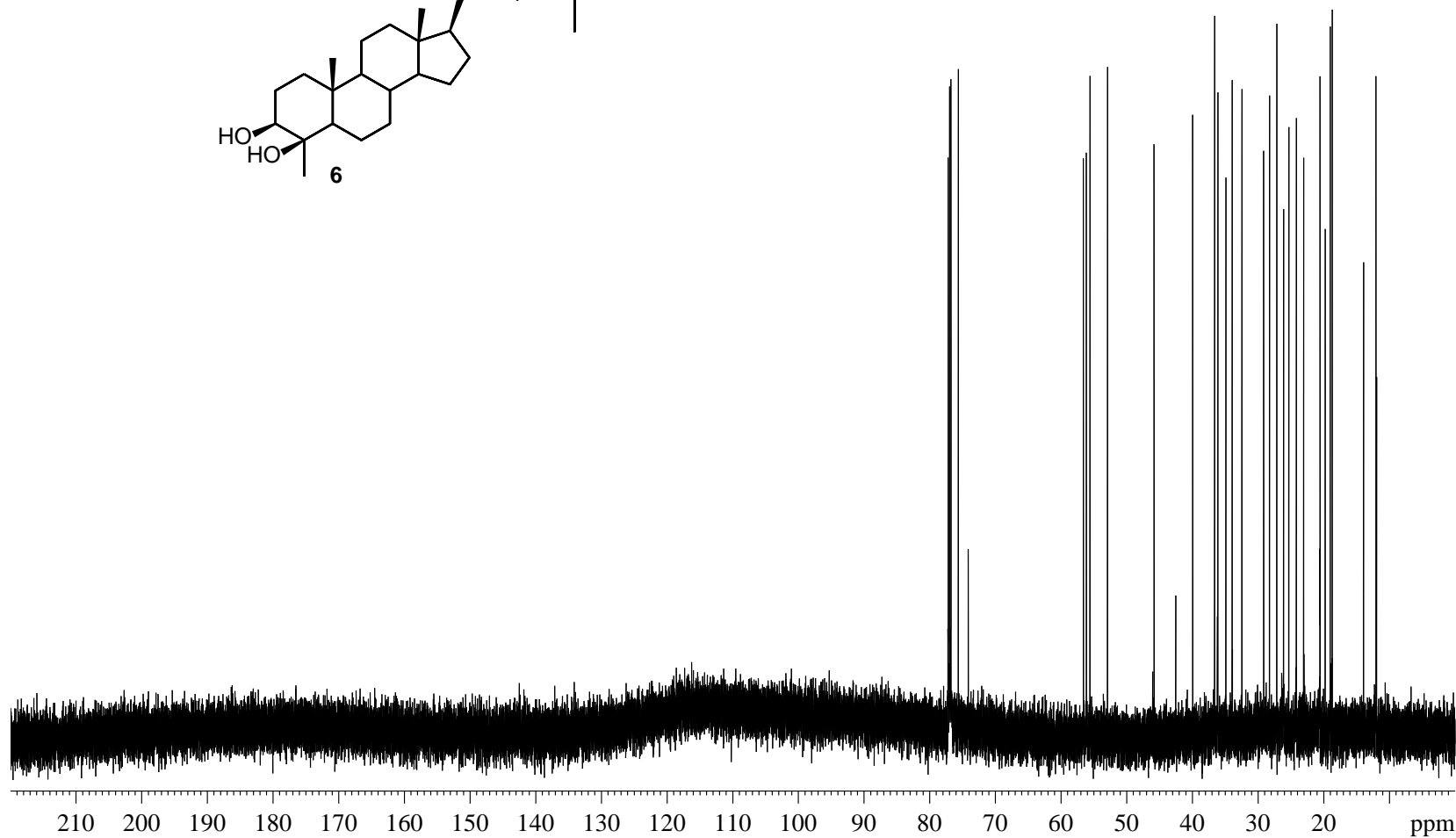
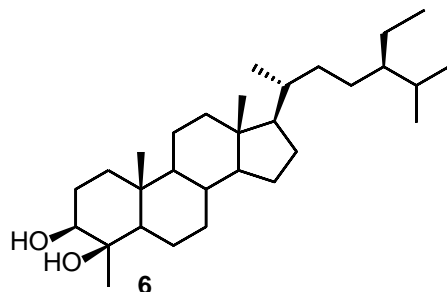
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



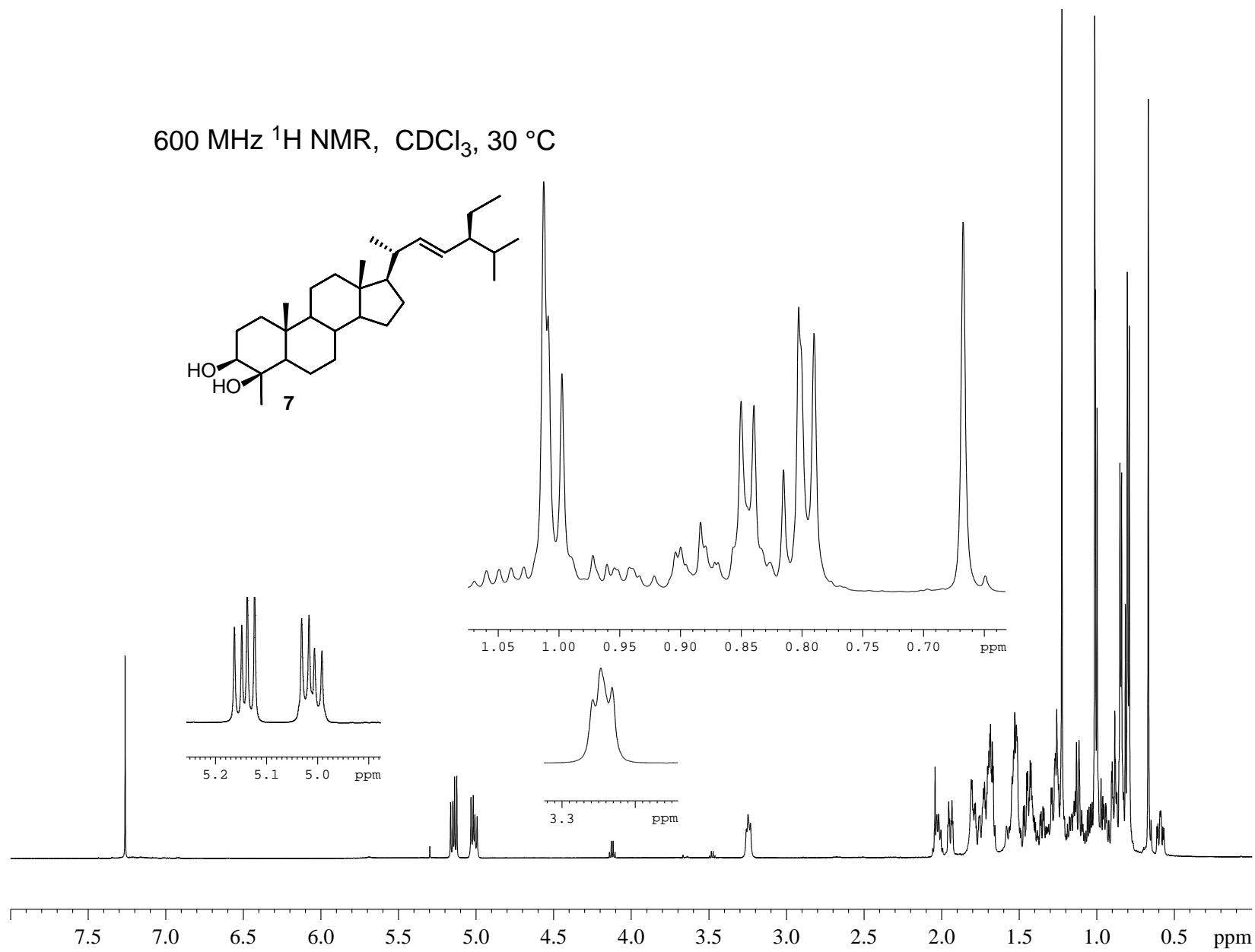
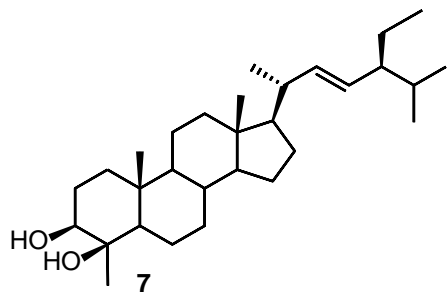
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



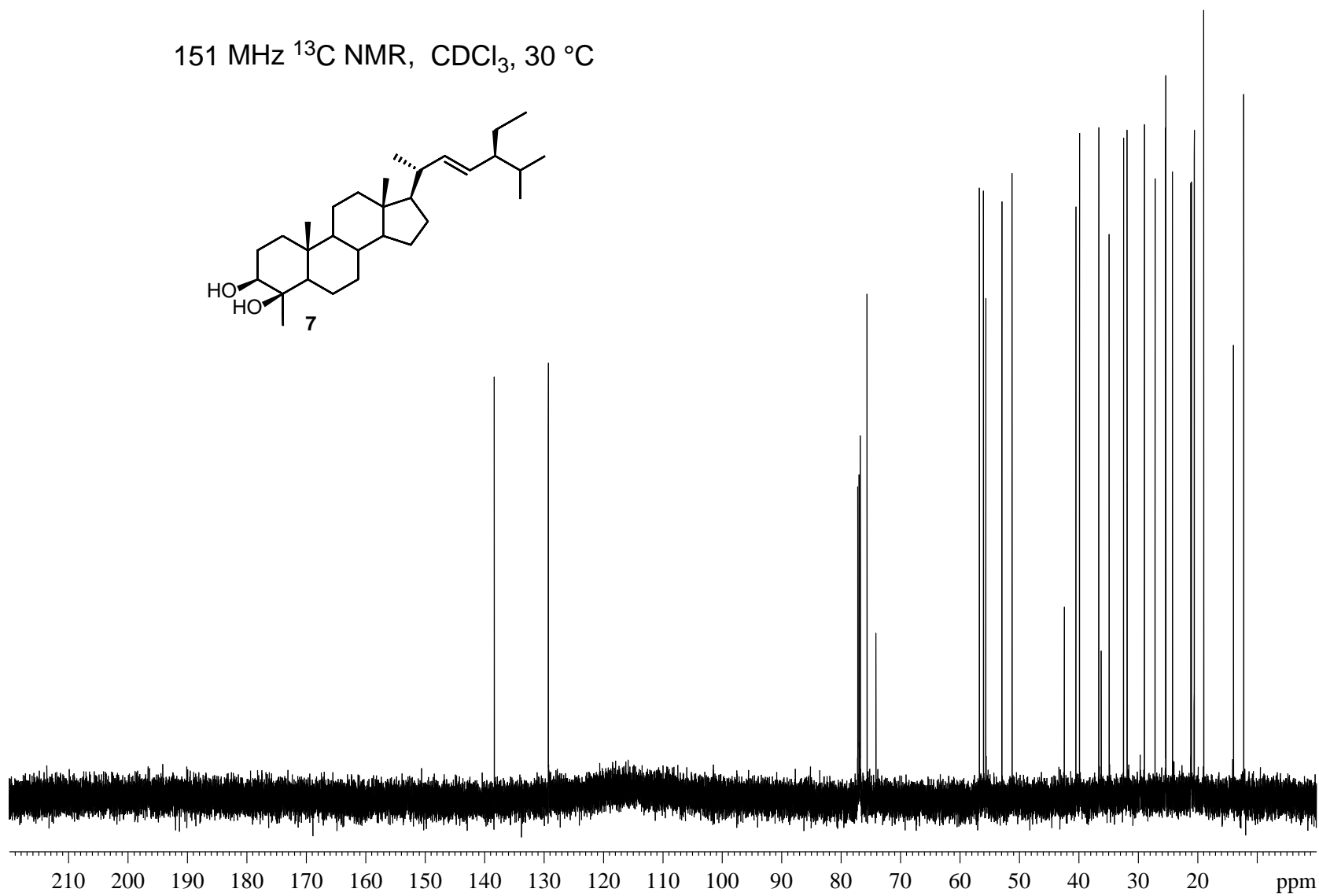
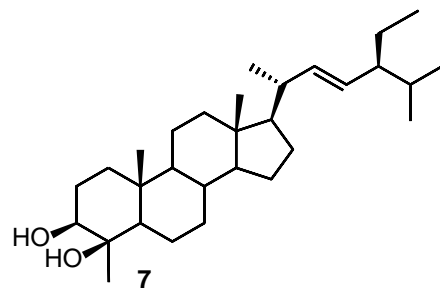
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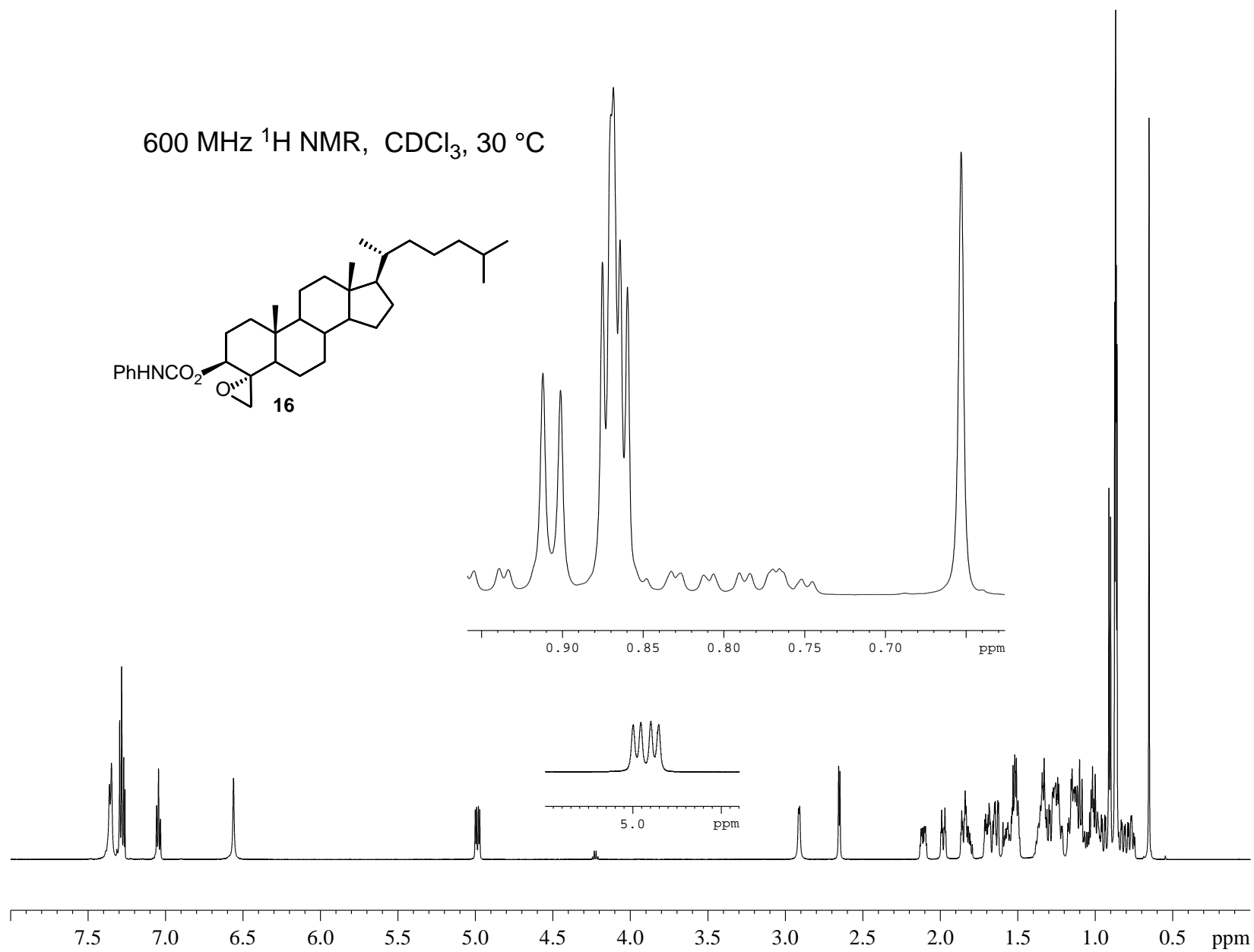
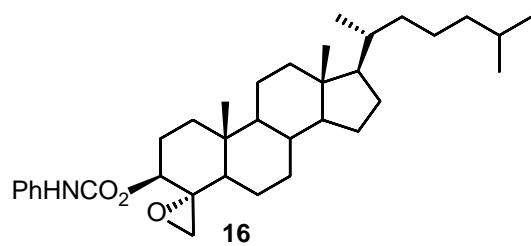
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



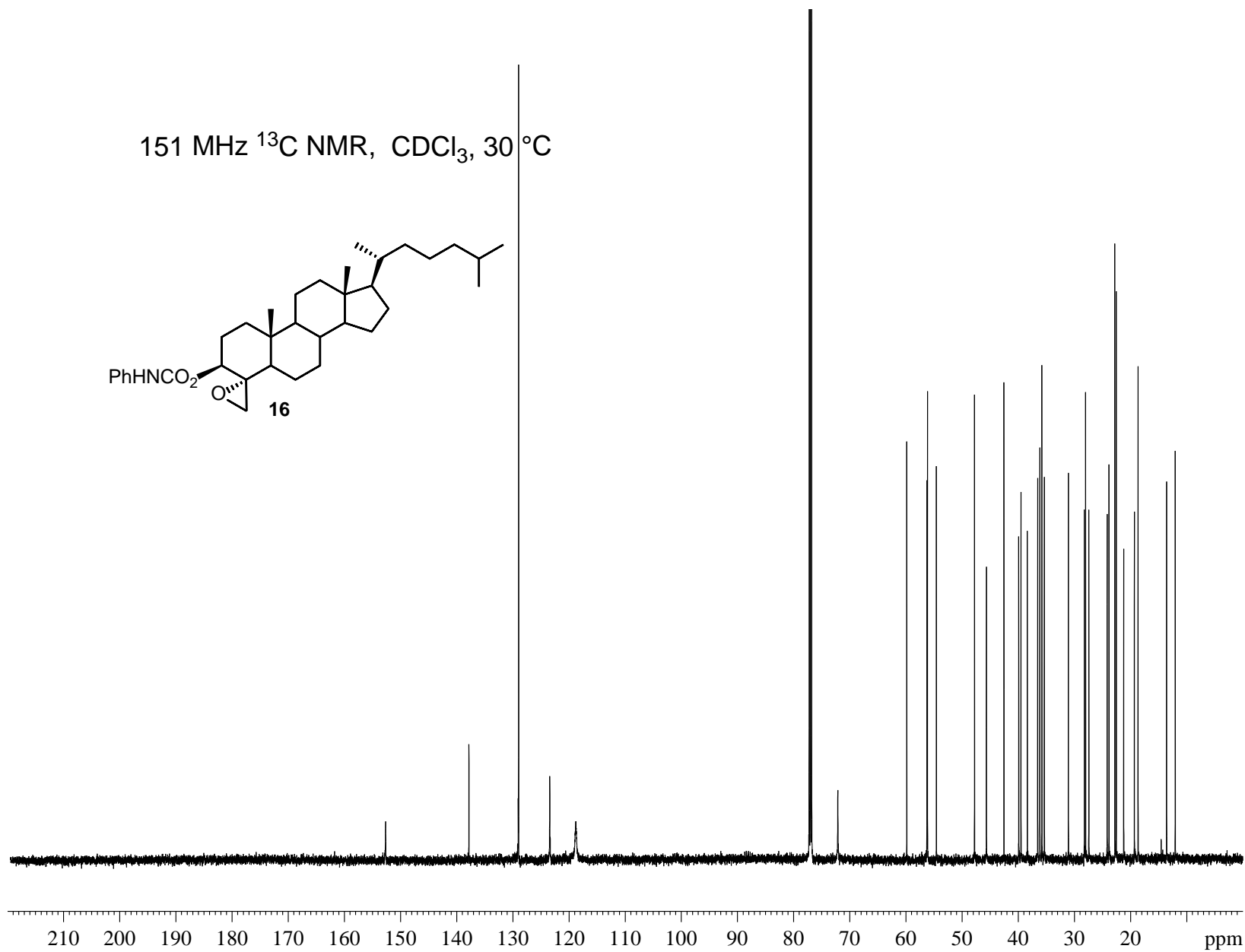
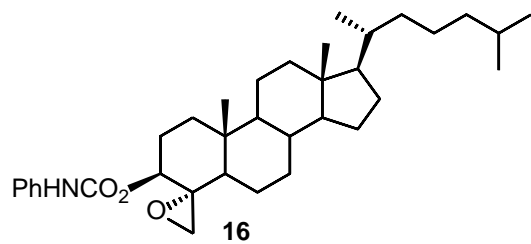
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$

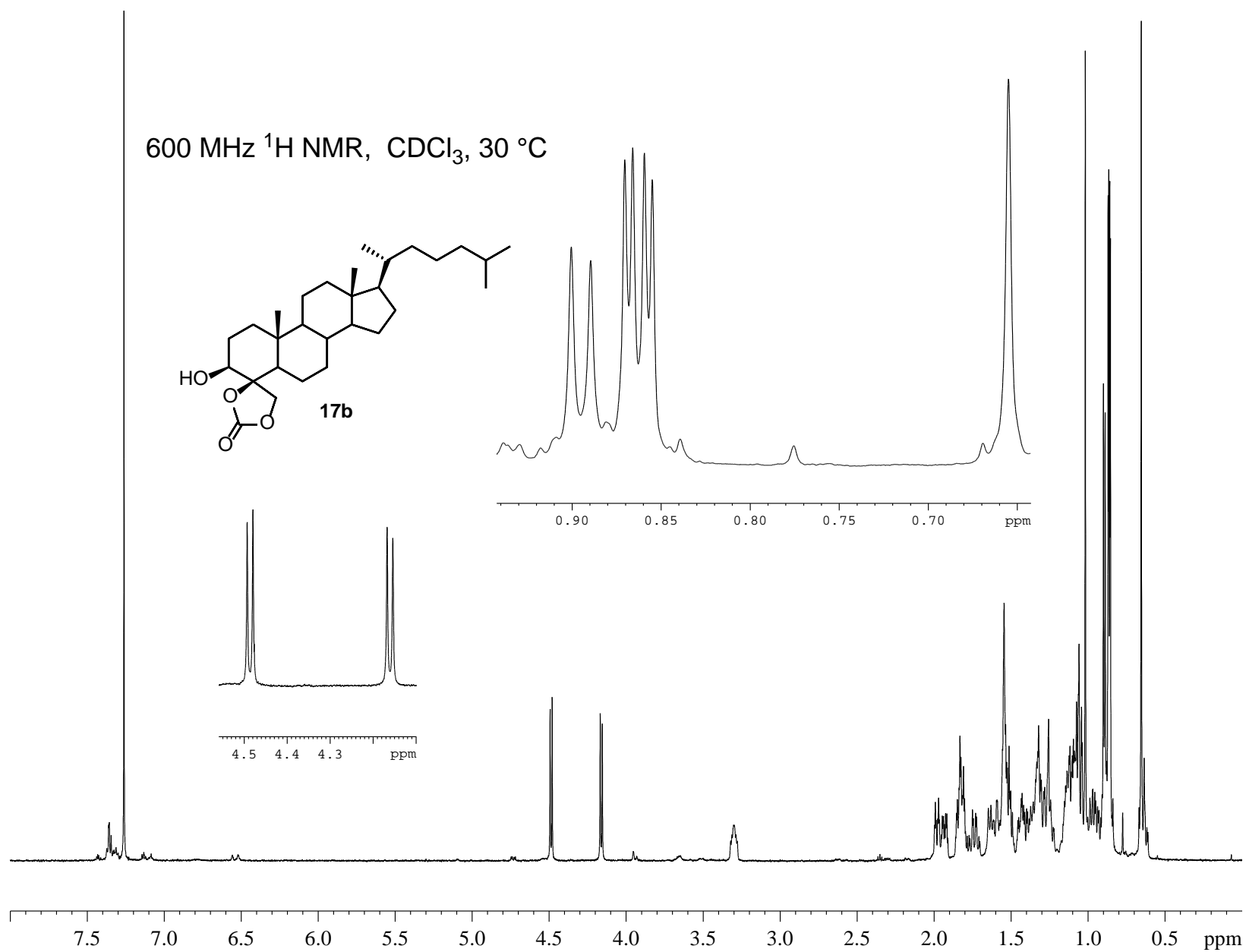


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

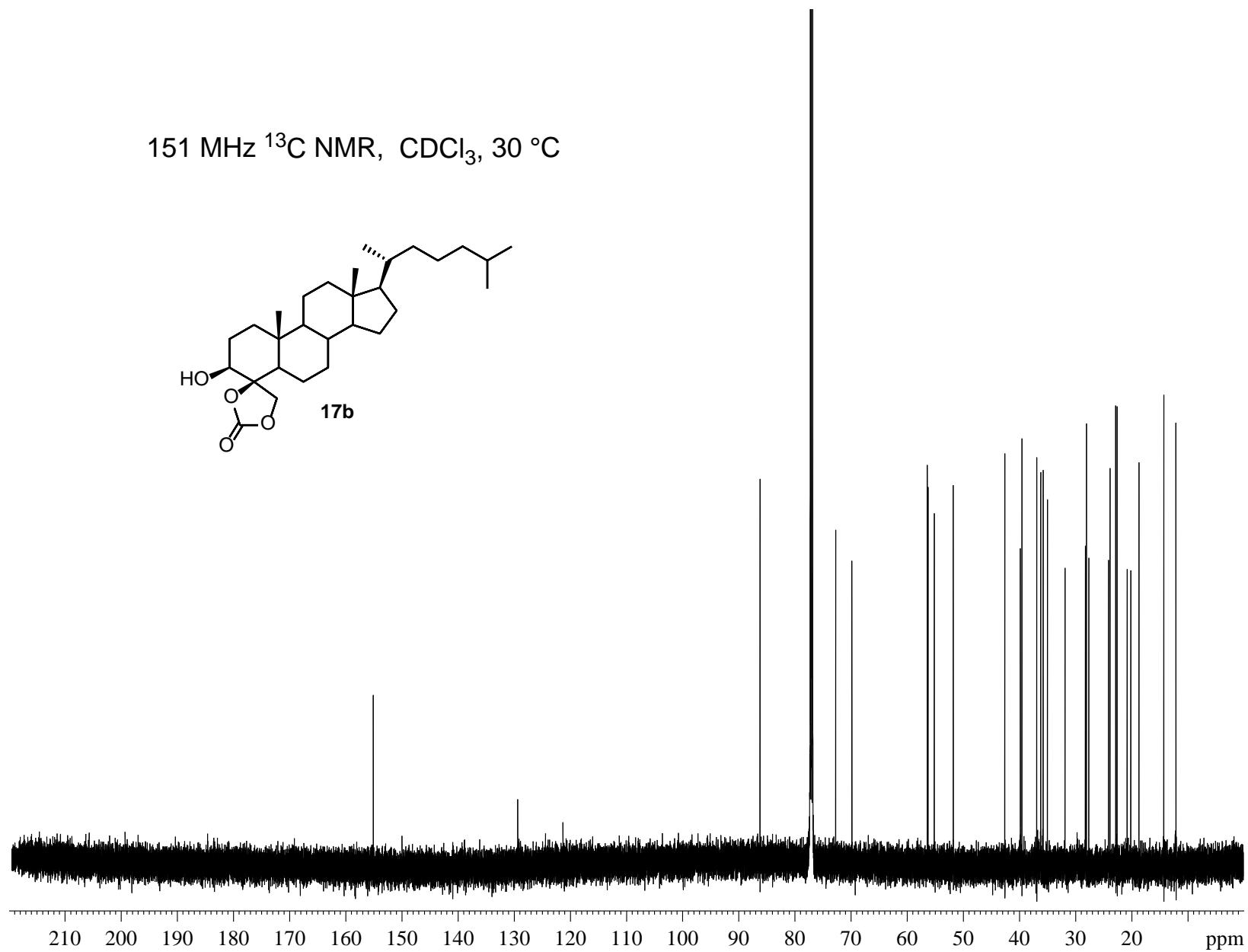
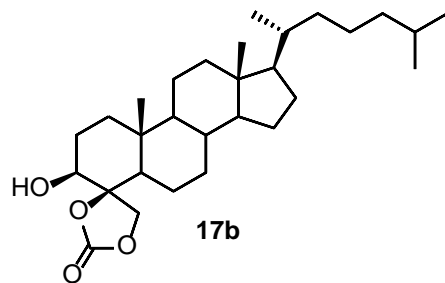


151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$

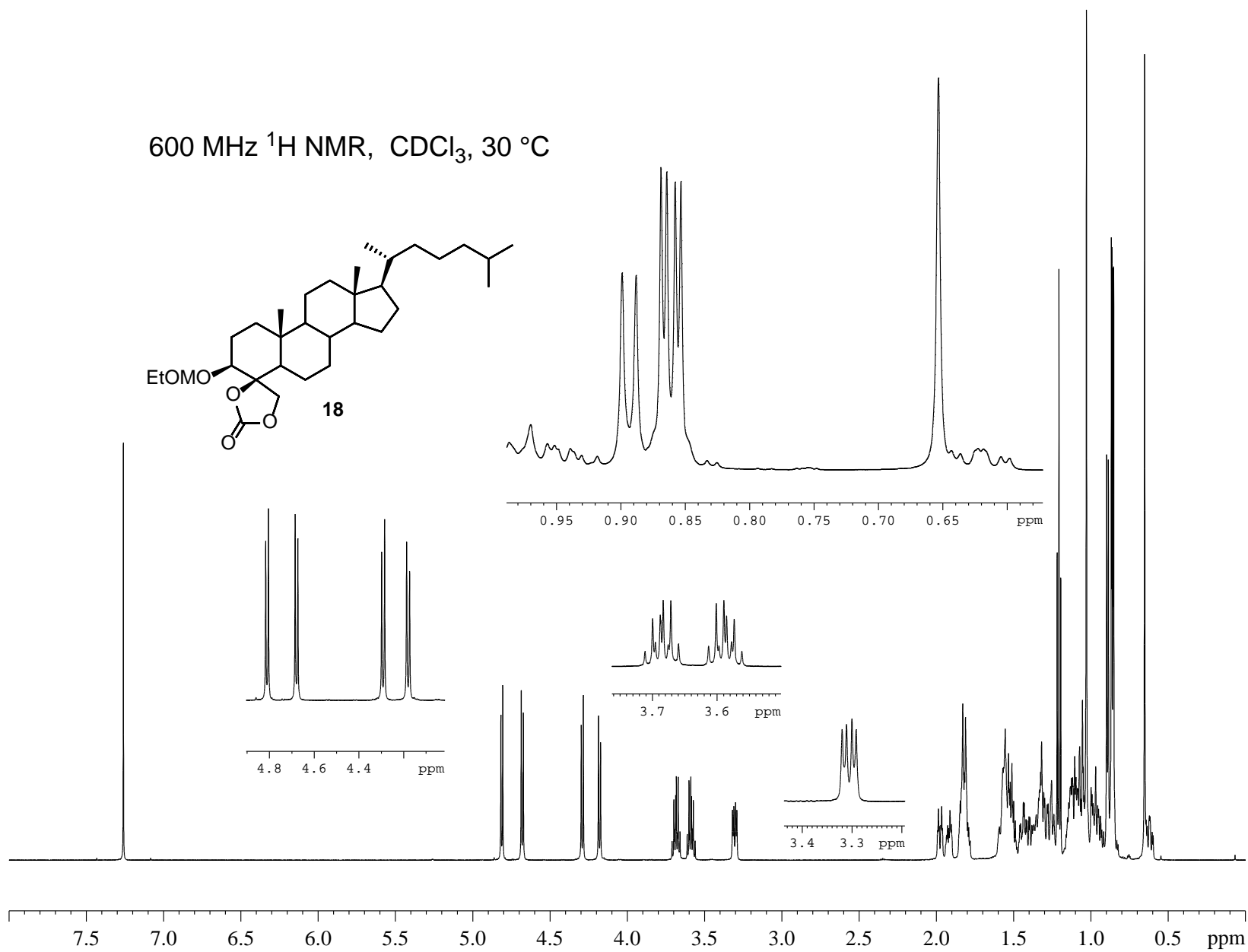




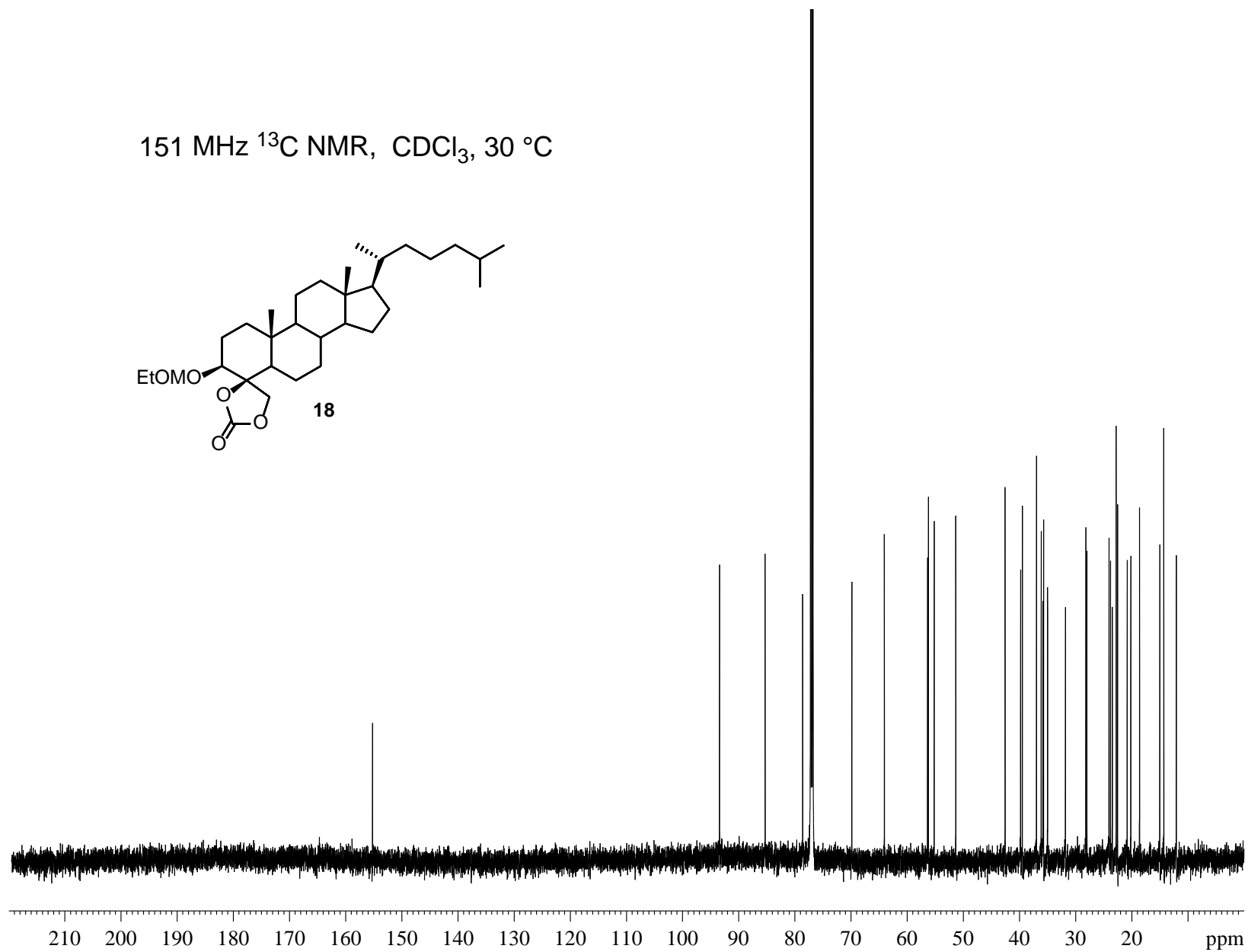
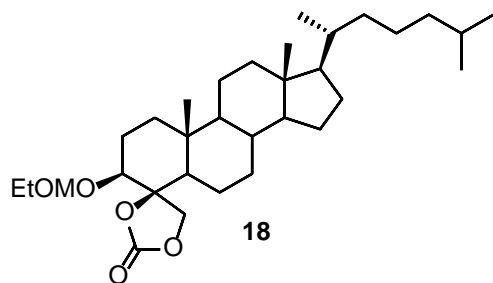
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



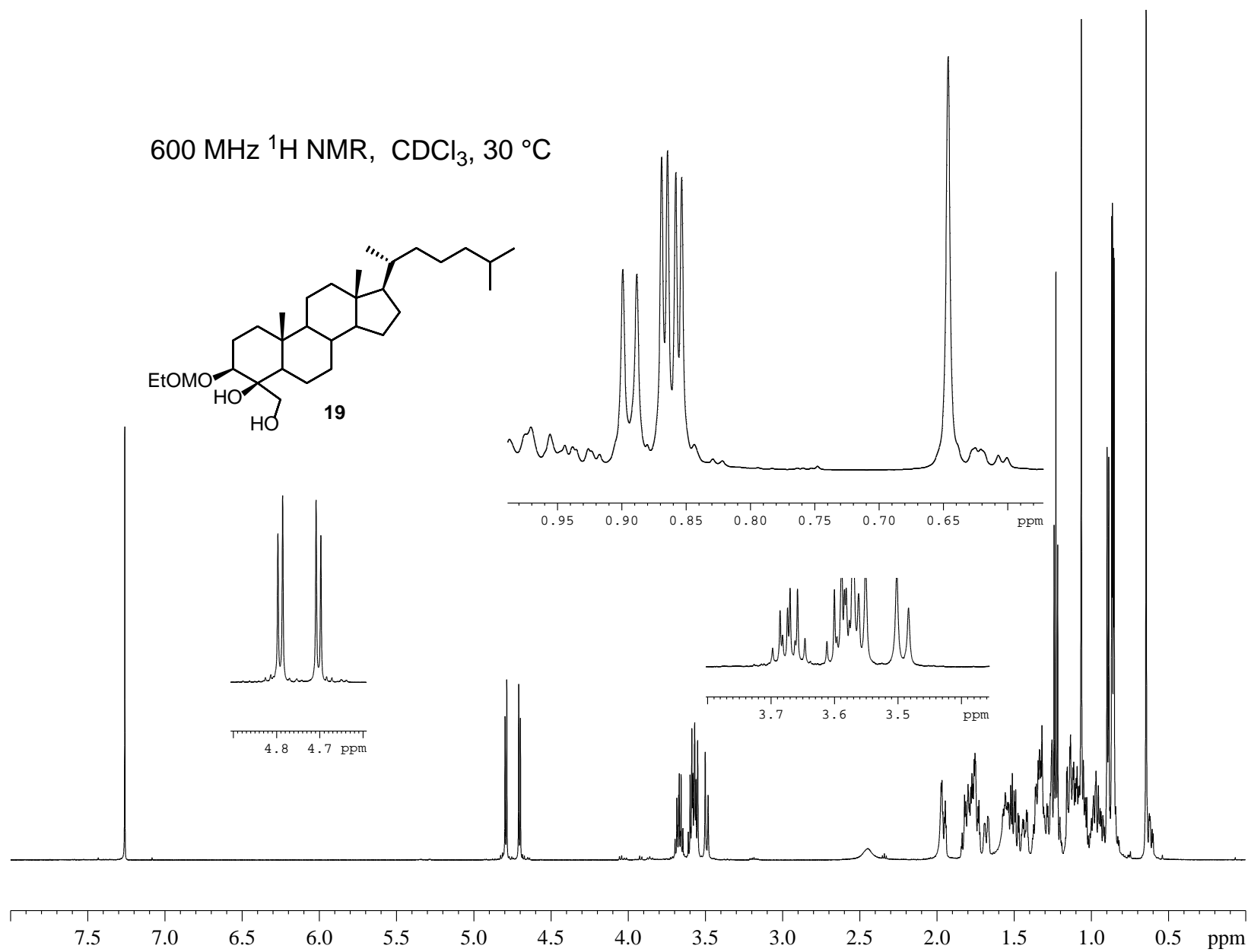
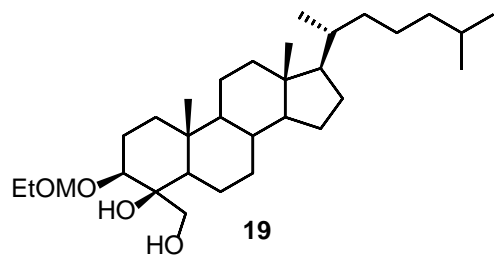
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



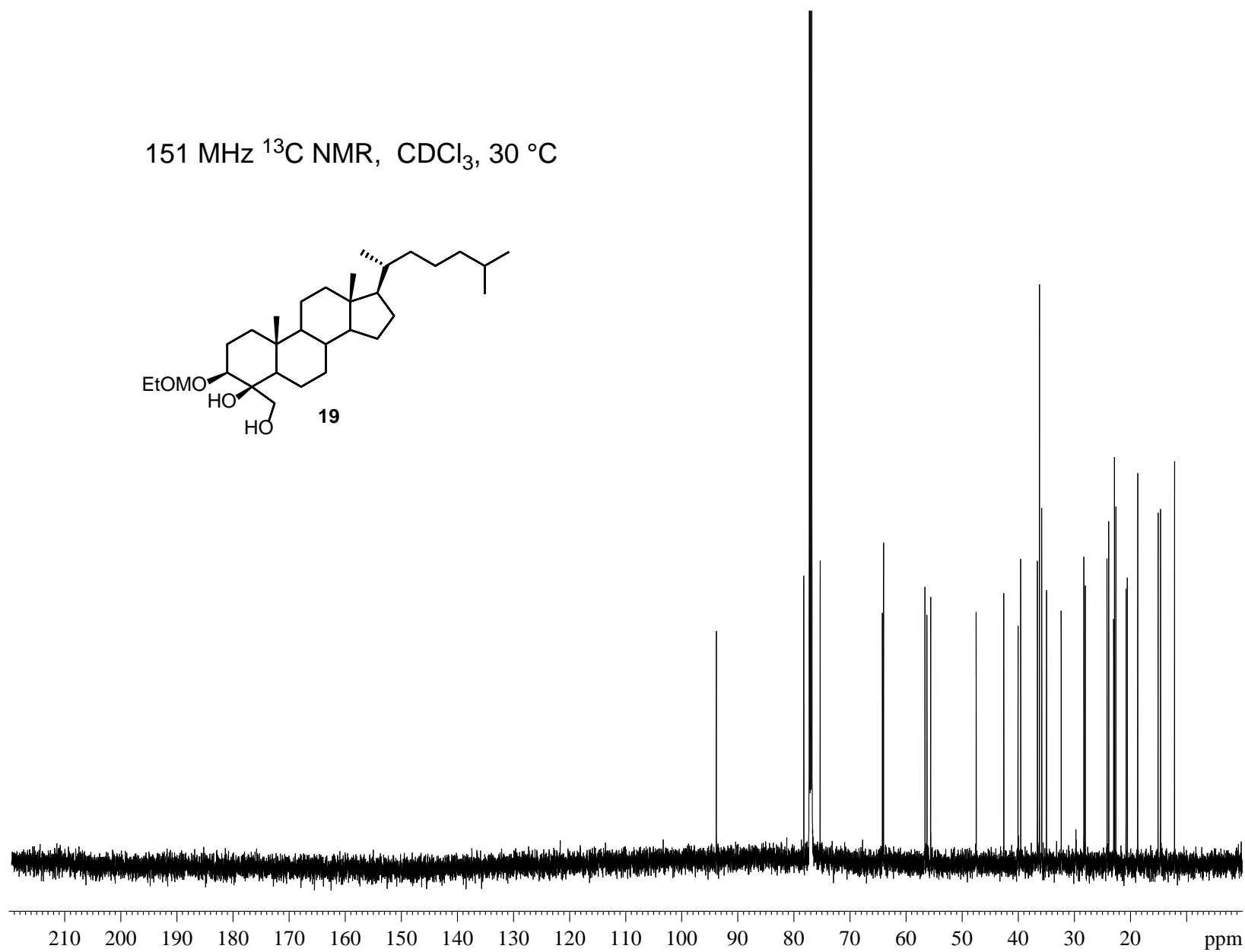
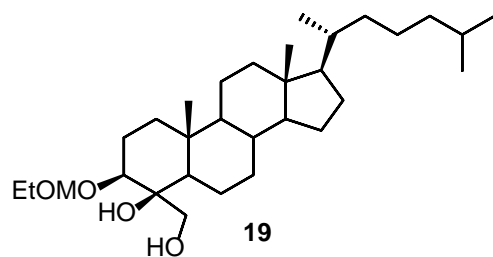
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$

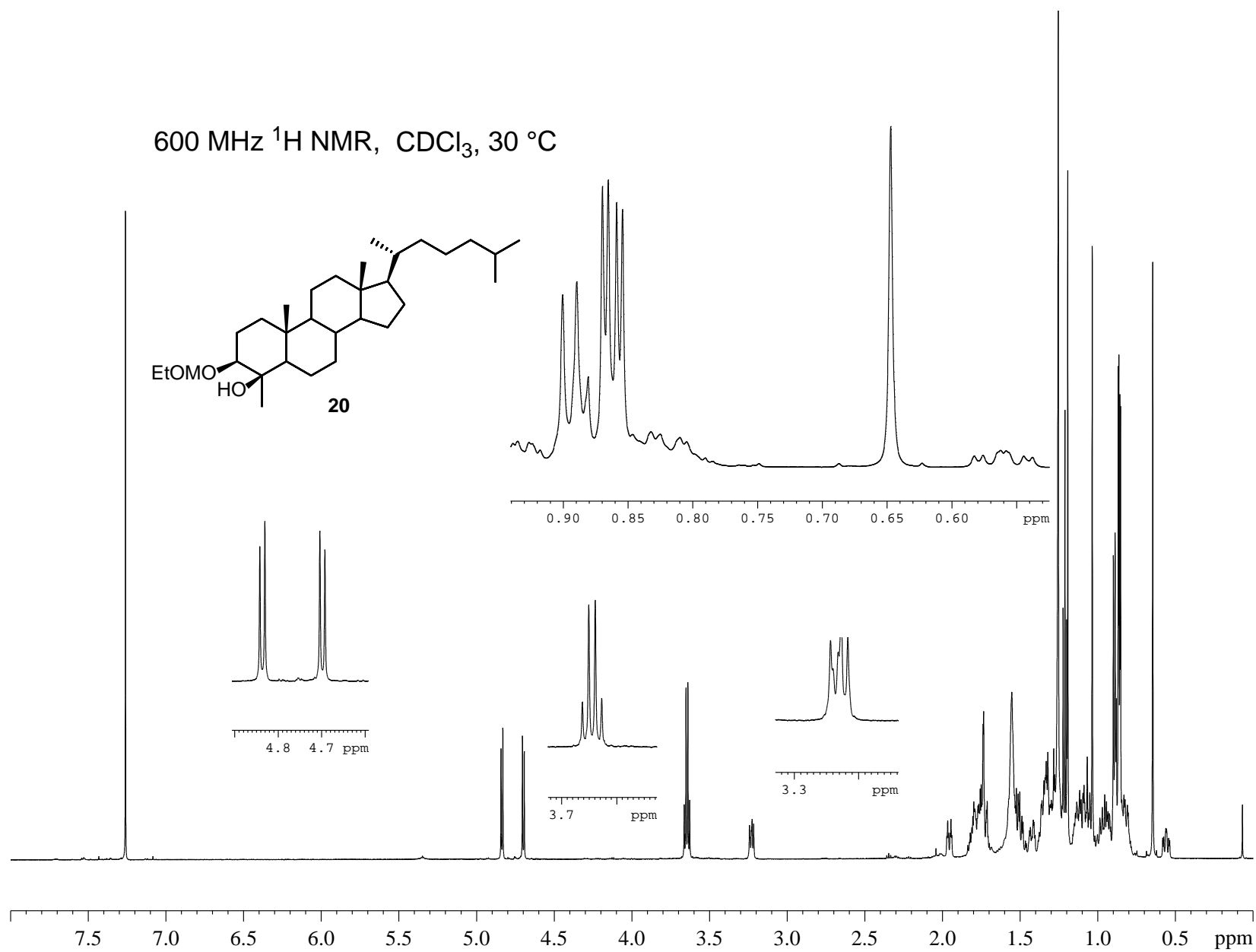


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

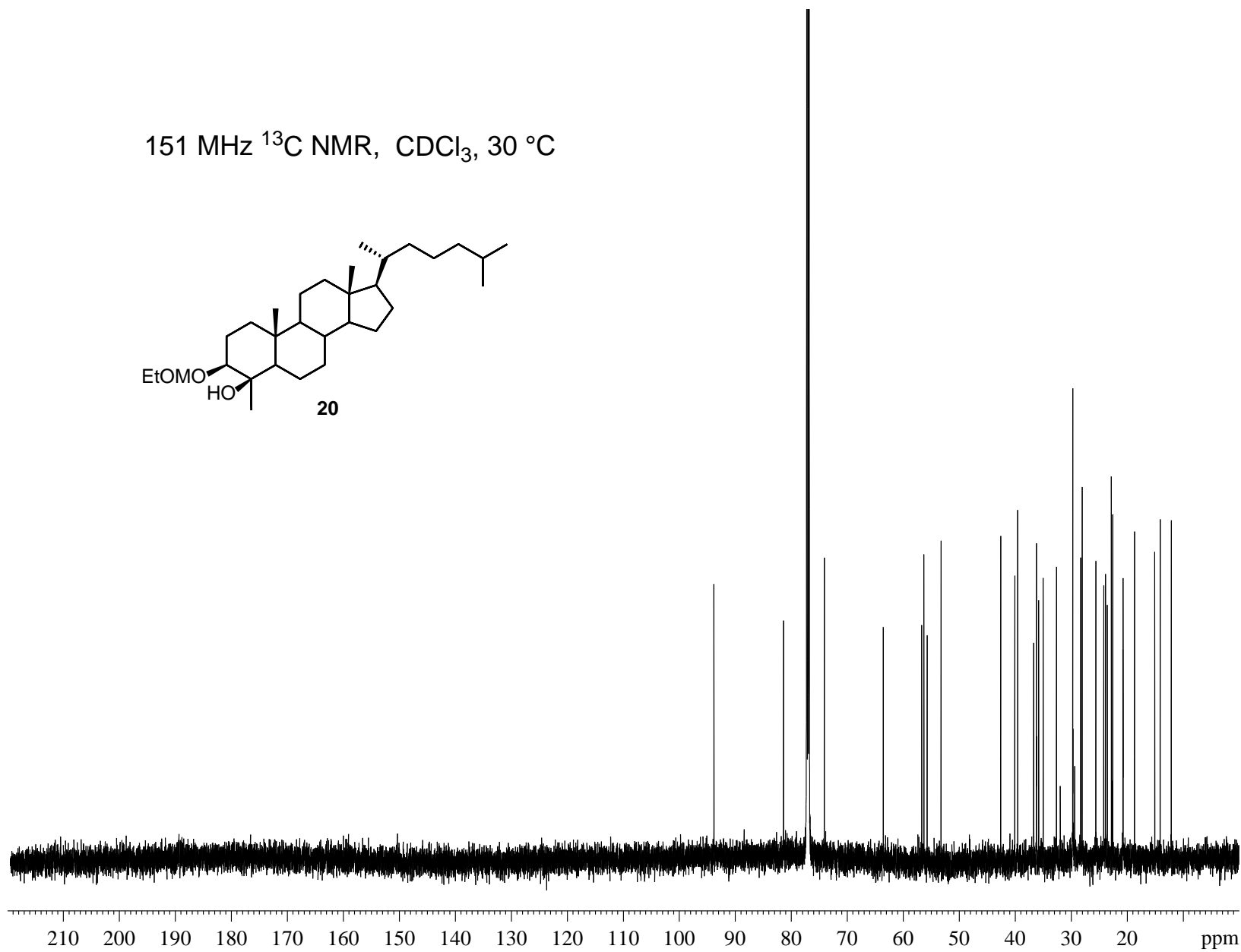
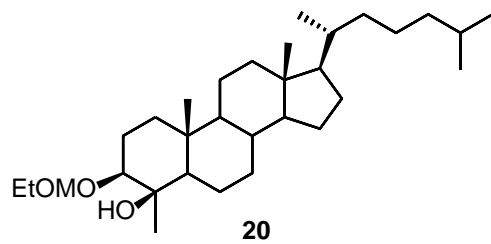


151 MHz ^{13}C NMR, CDCl_3 , 30 °C

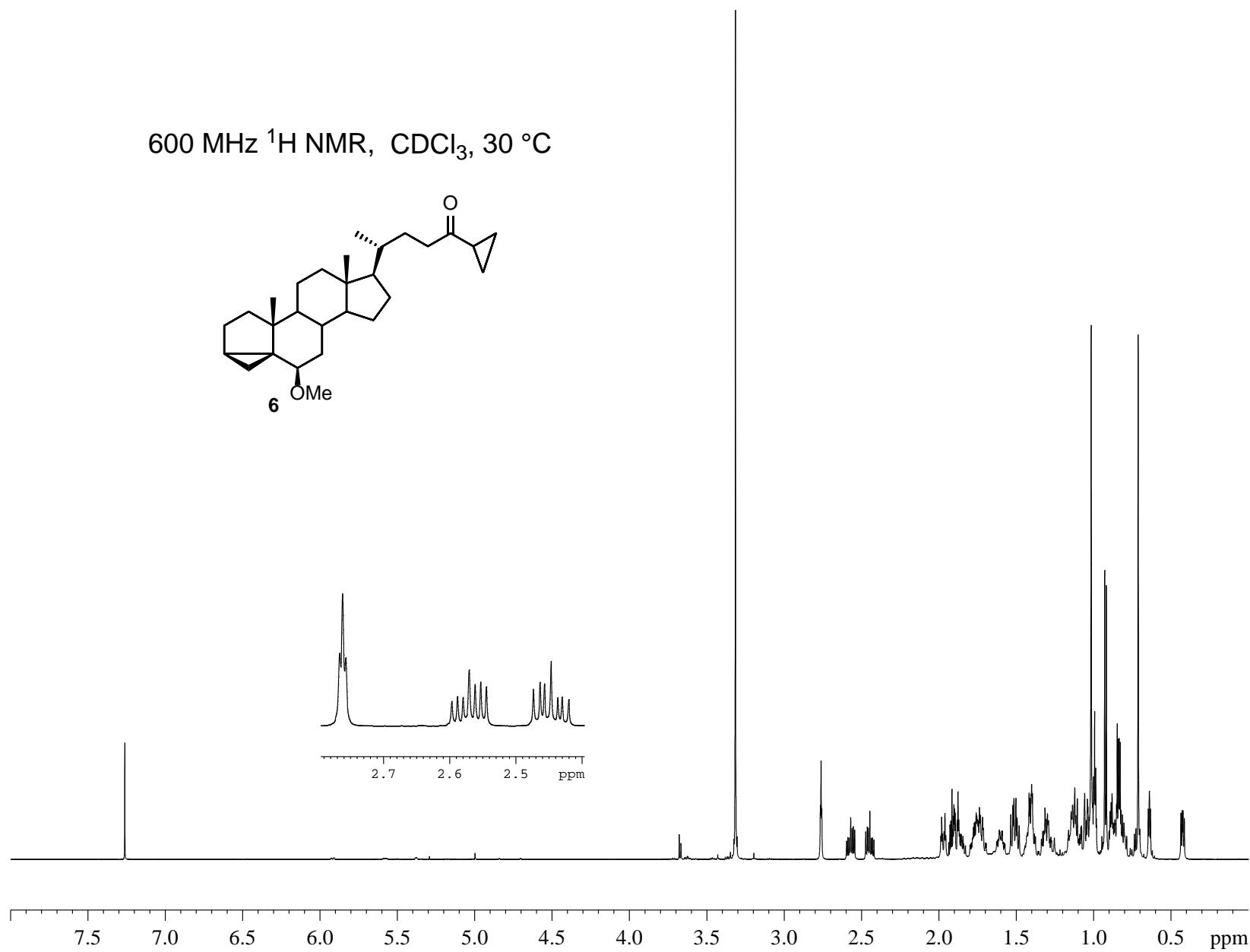
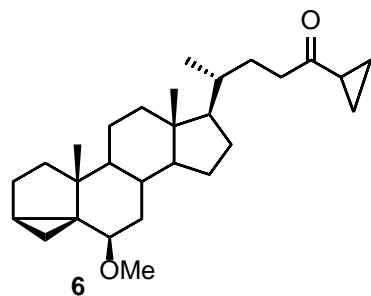




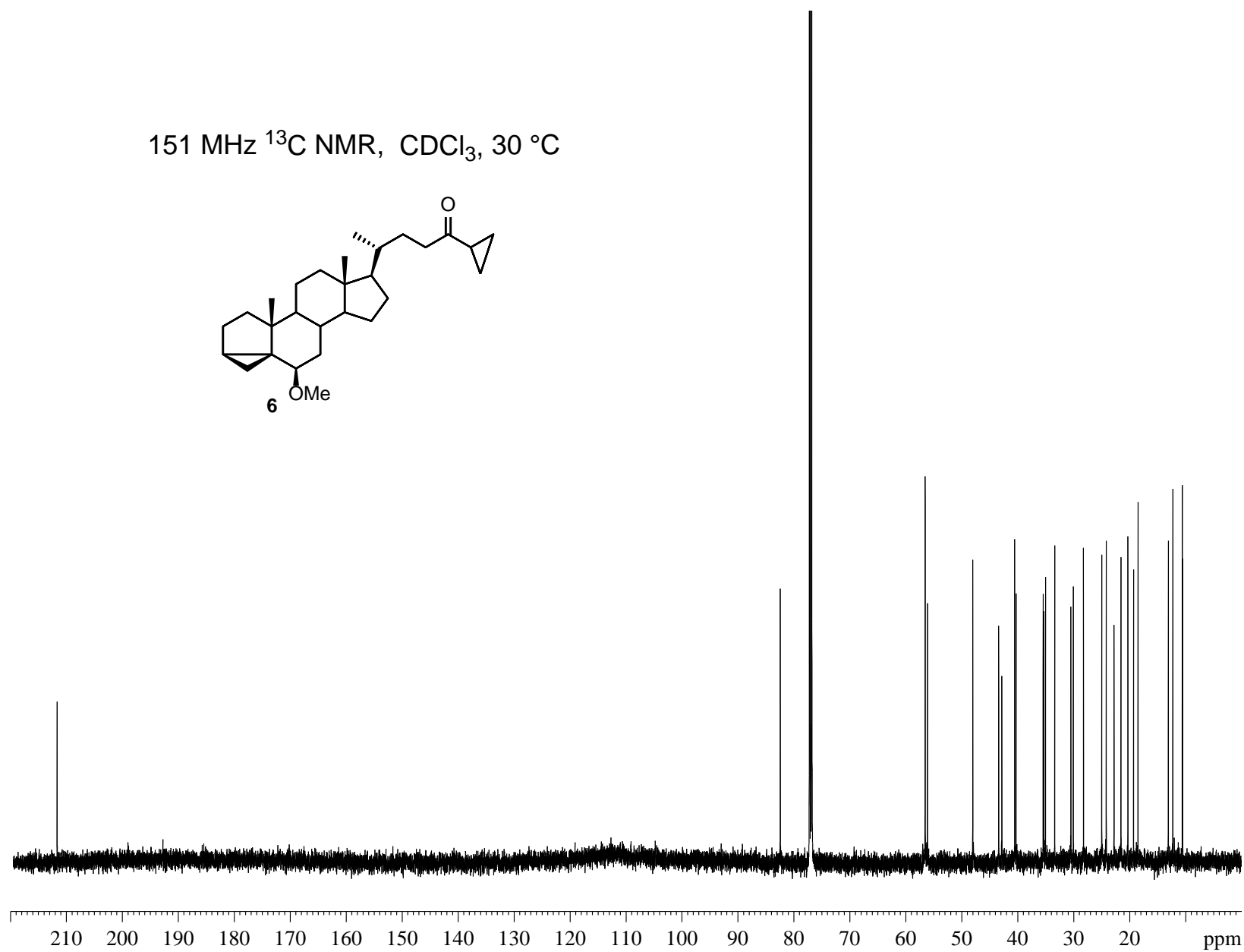
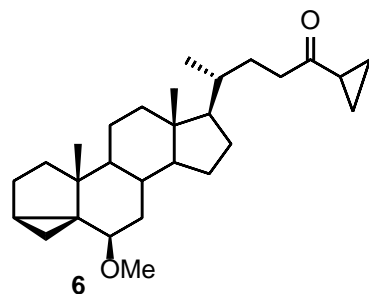
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



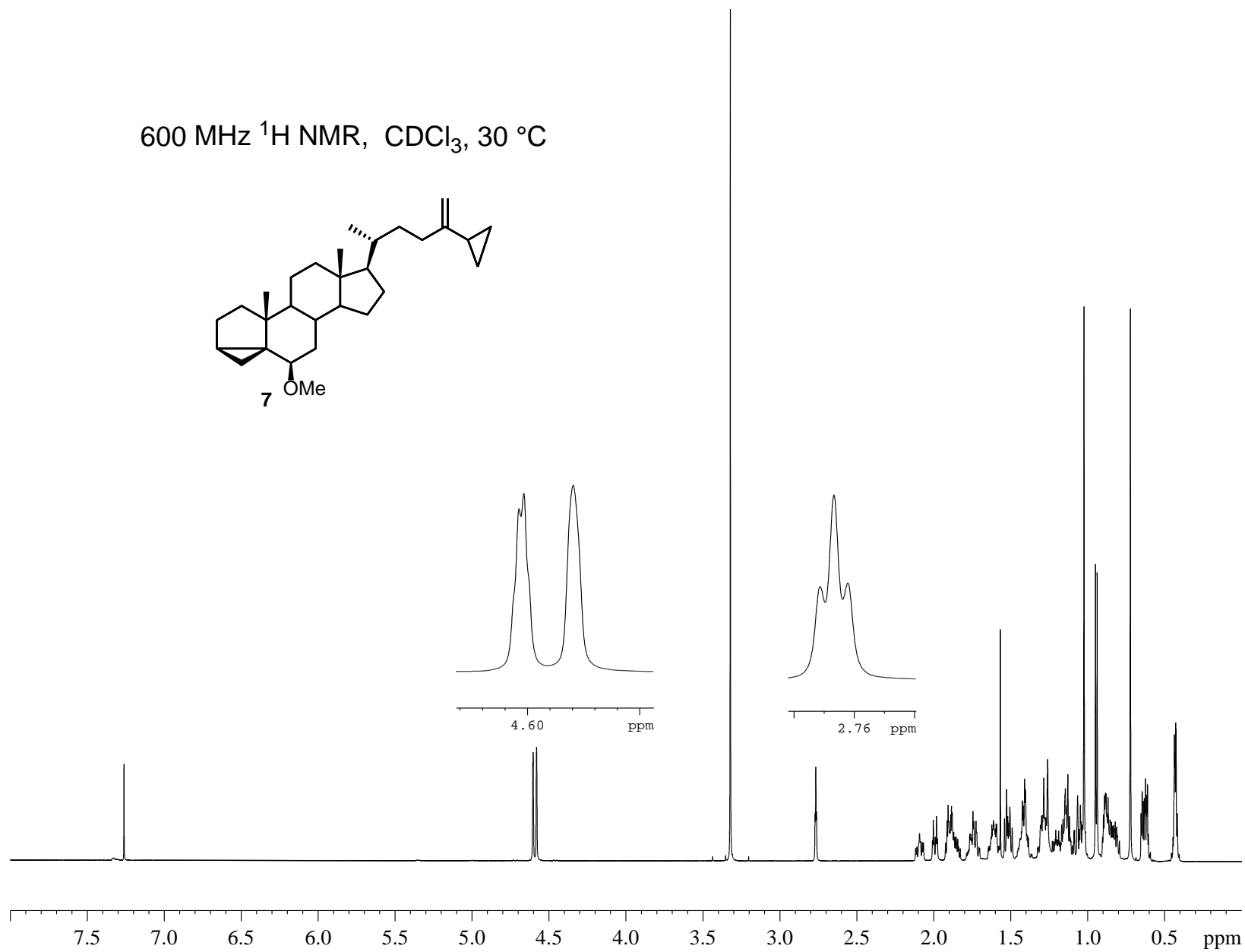
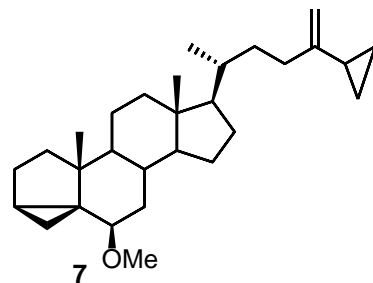
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



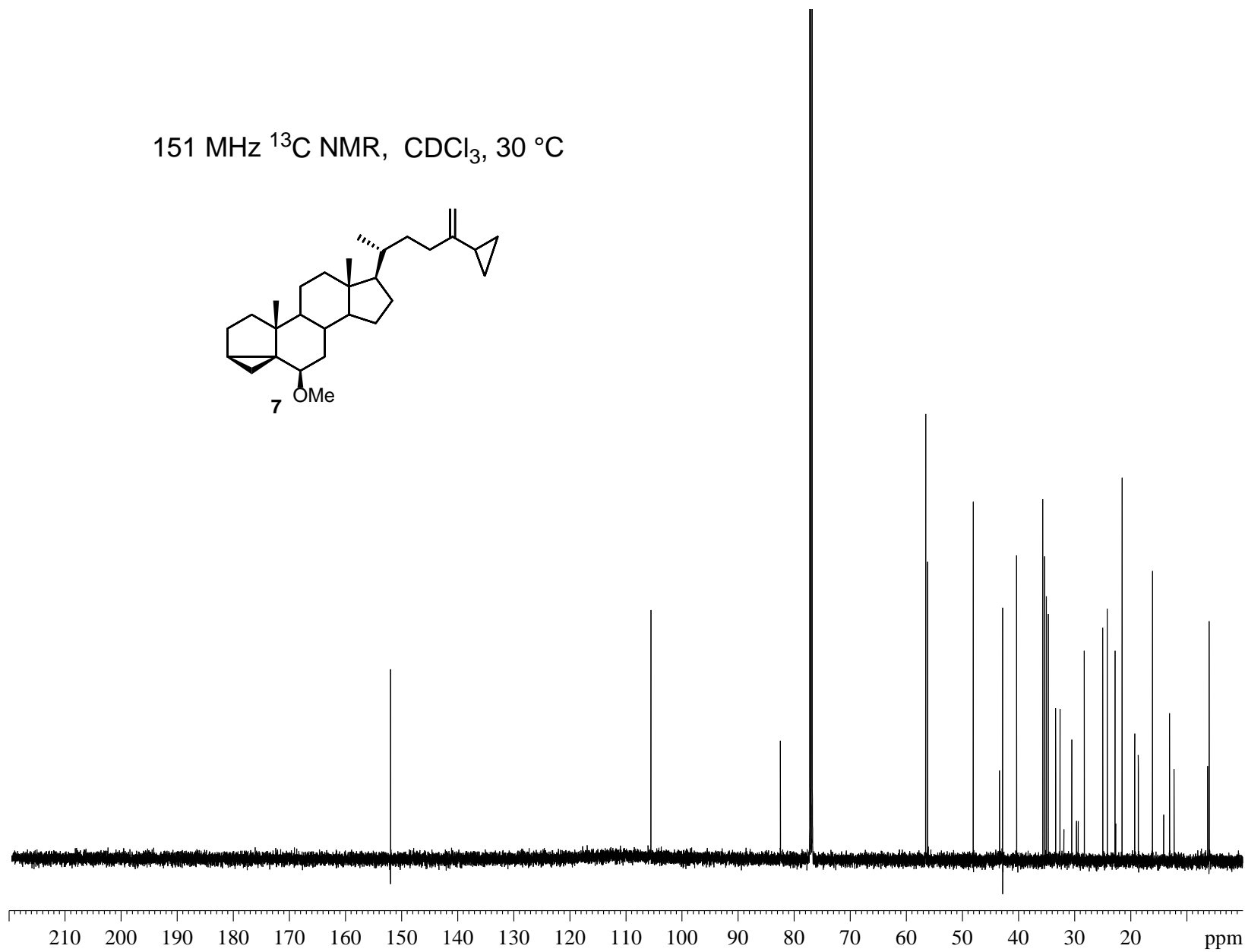
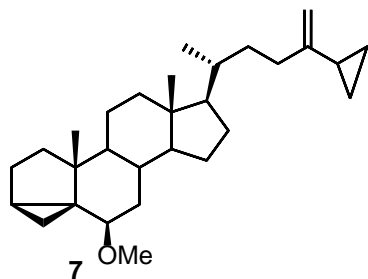
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



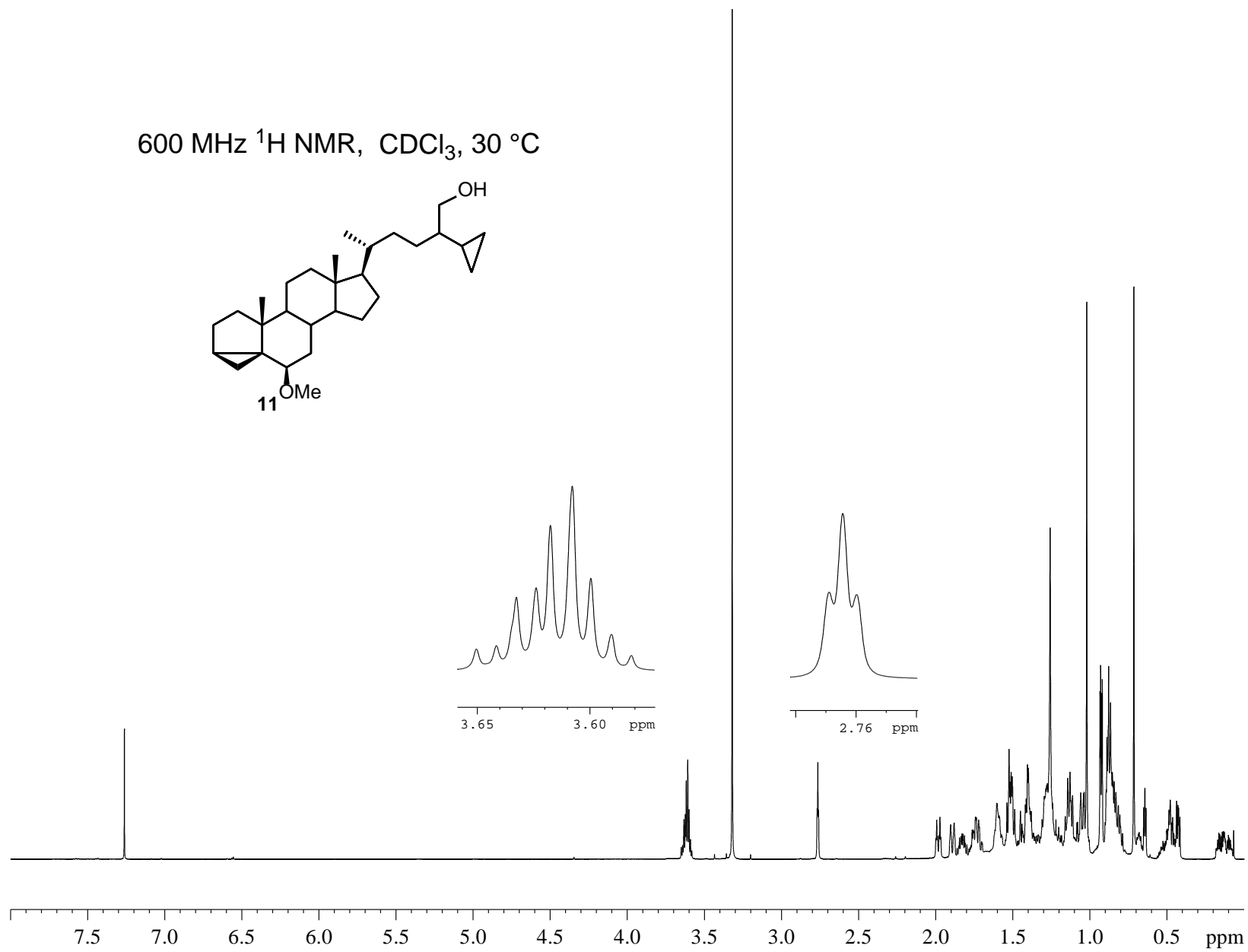
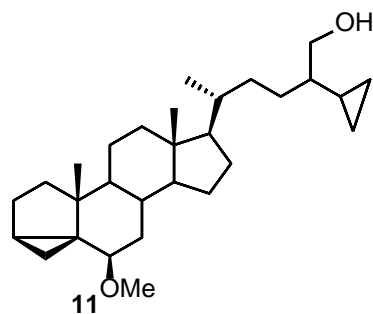
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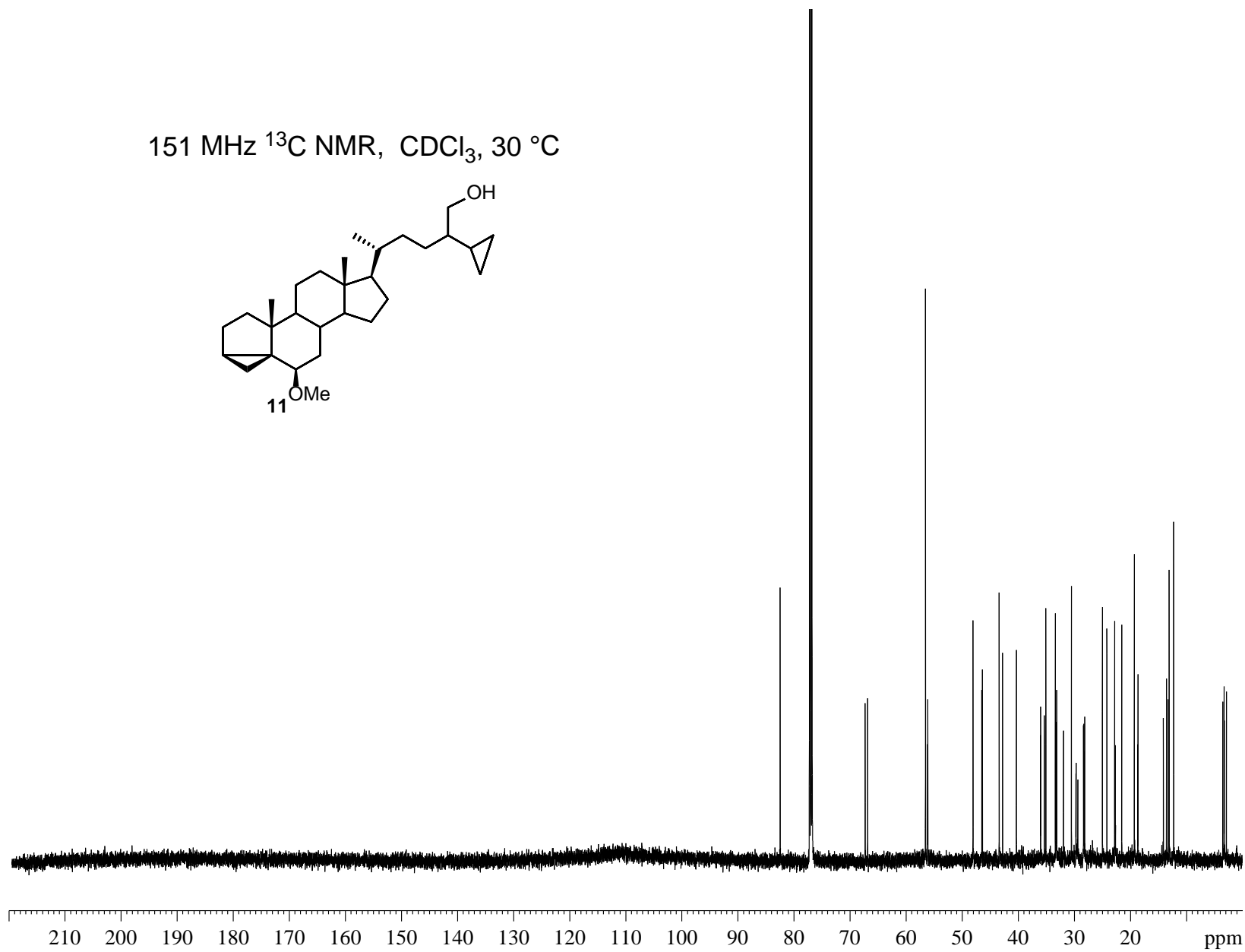
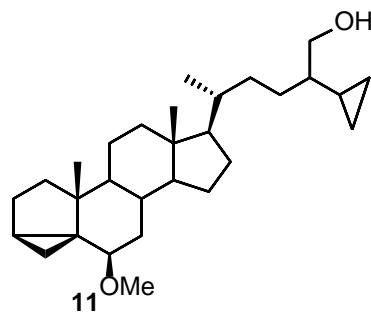
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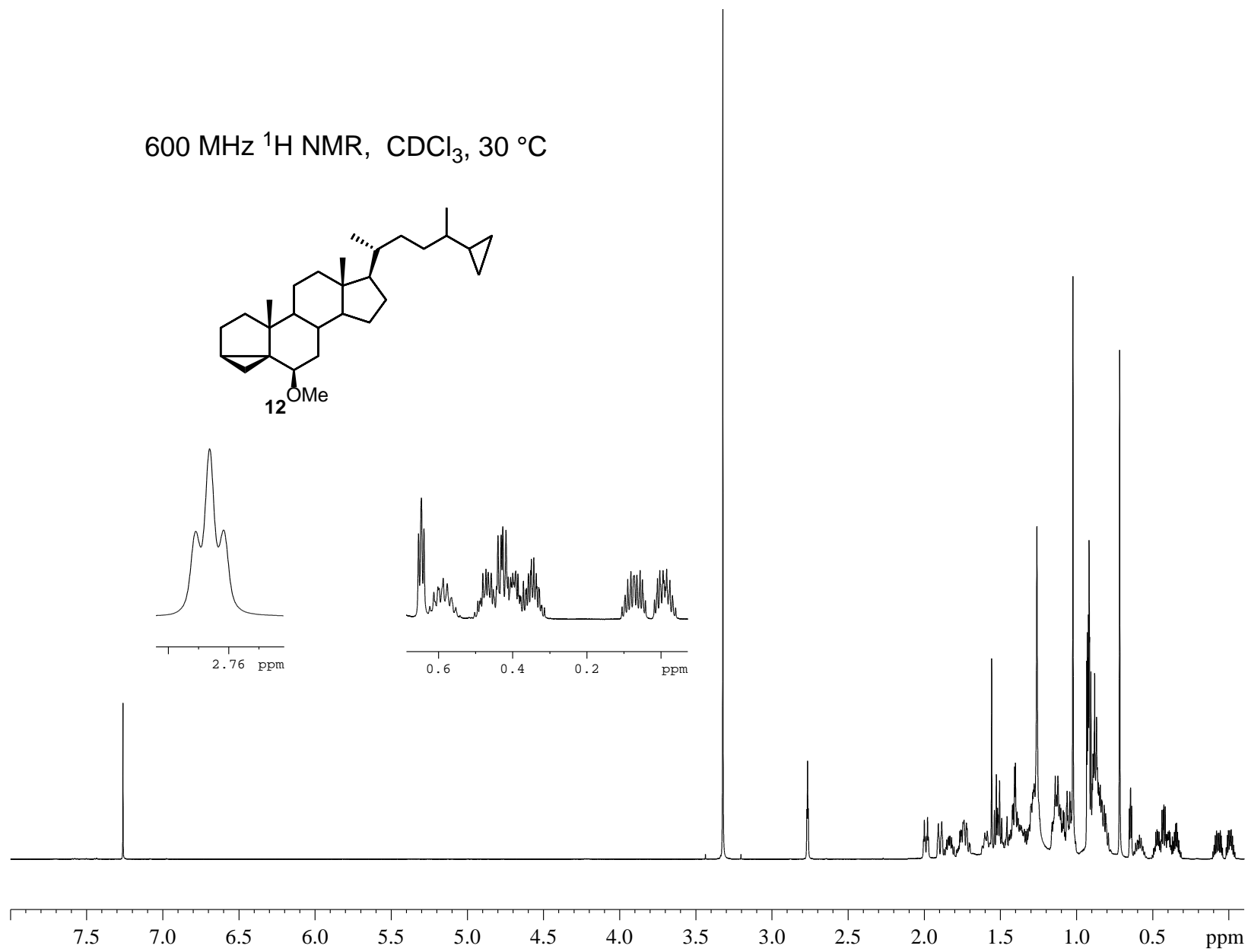
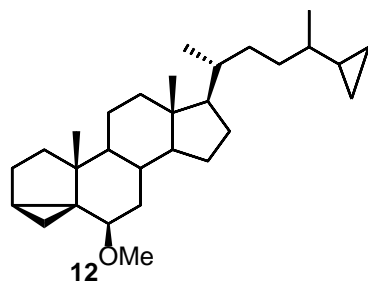
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



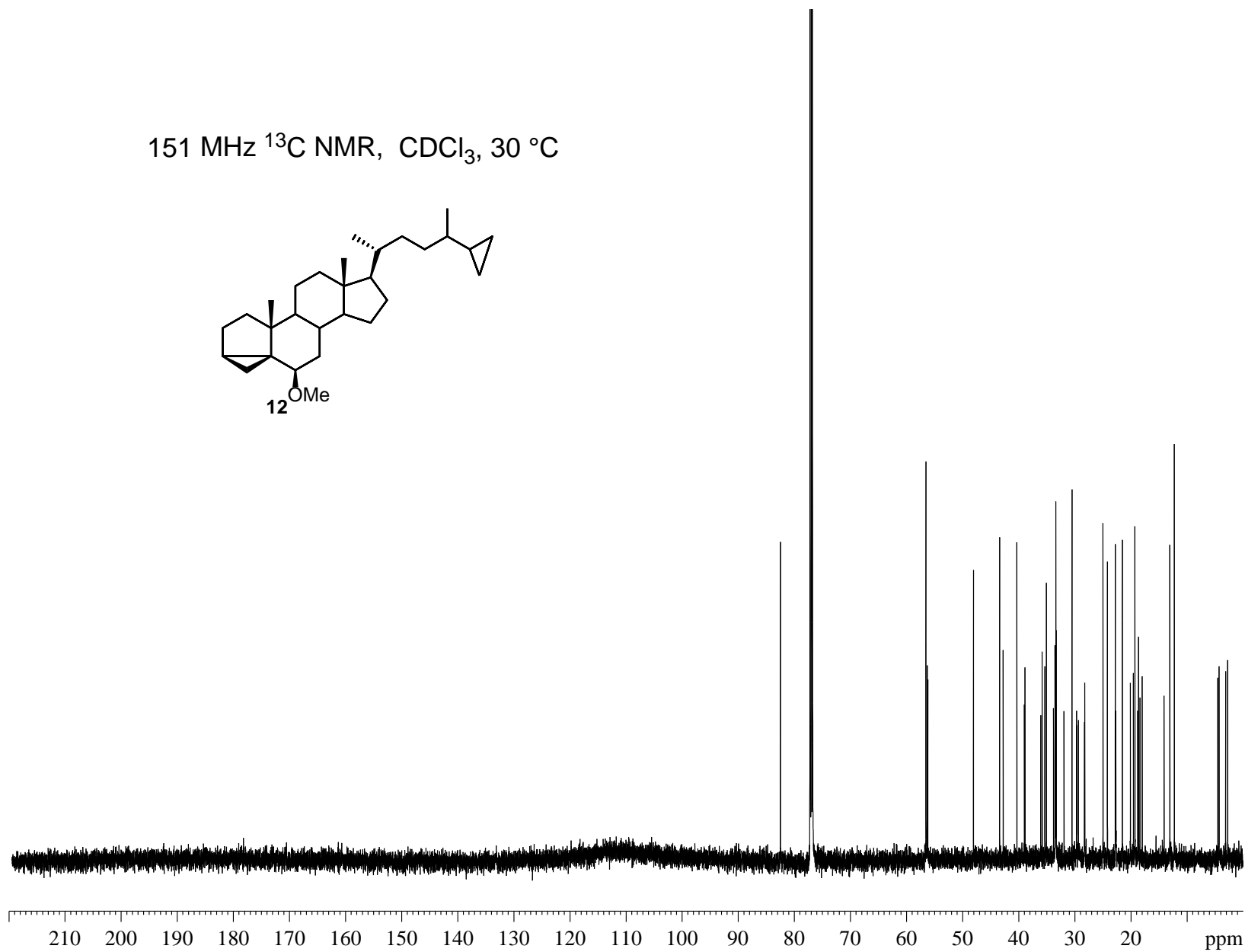
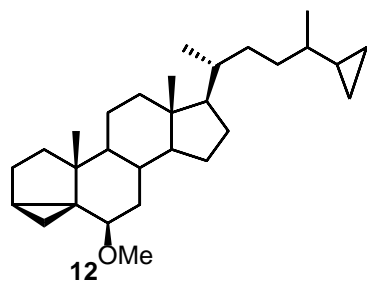
151 MHz ^{13}C NMR, CDCl_3 , 30 $^{\circ}\text{C}$

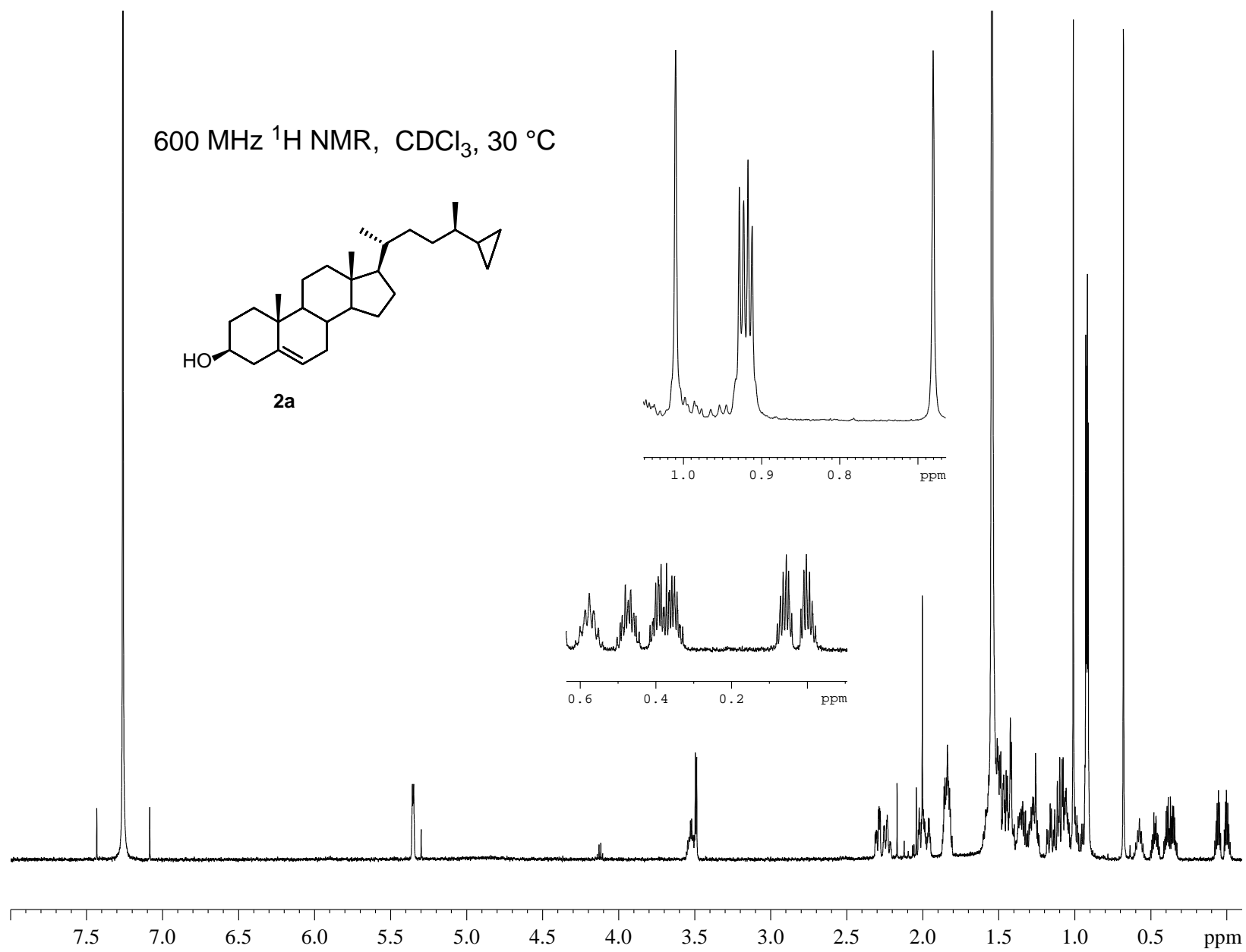


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$





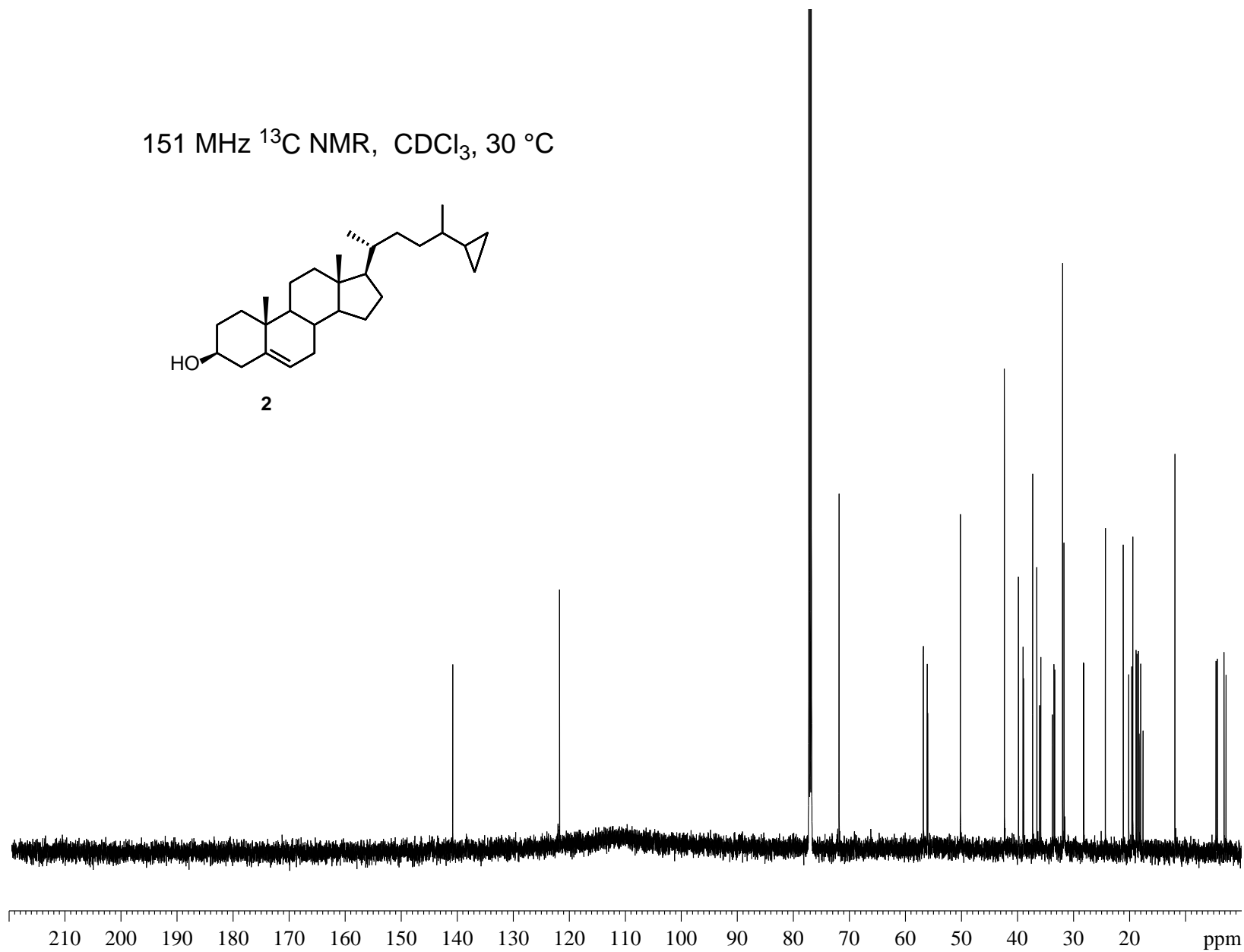
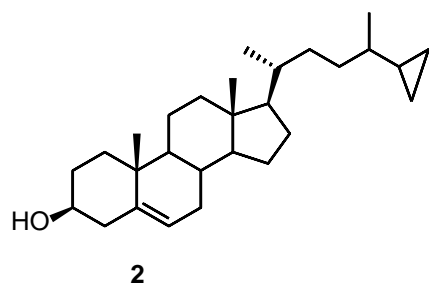
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2b

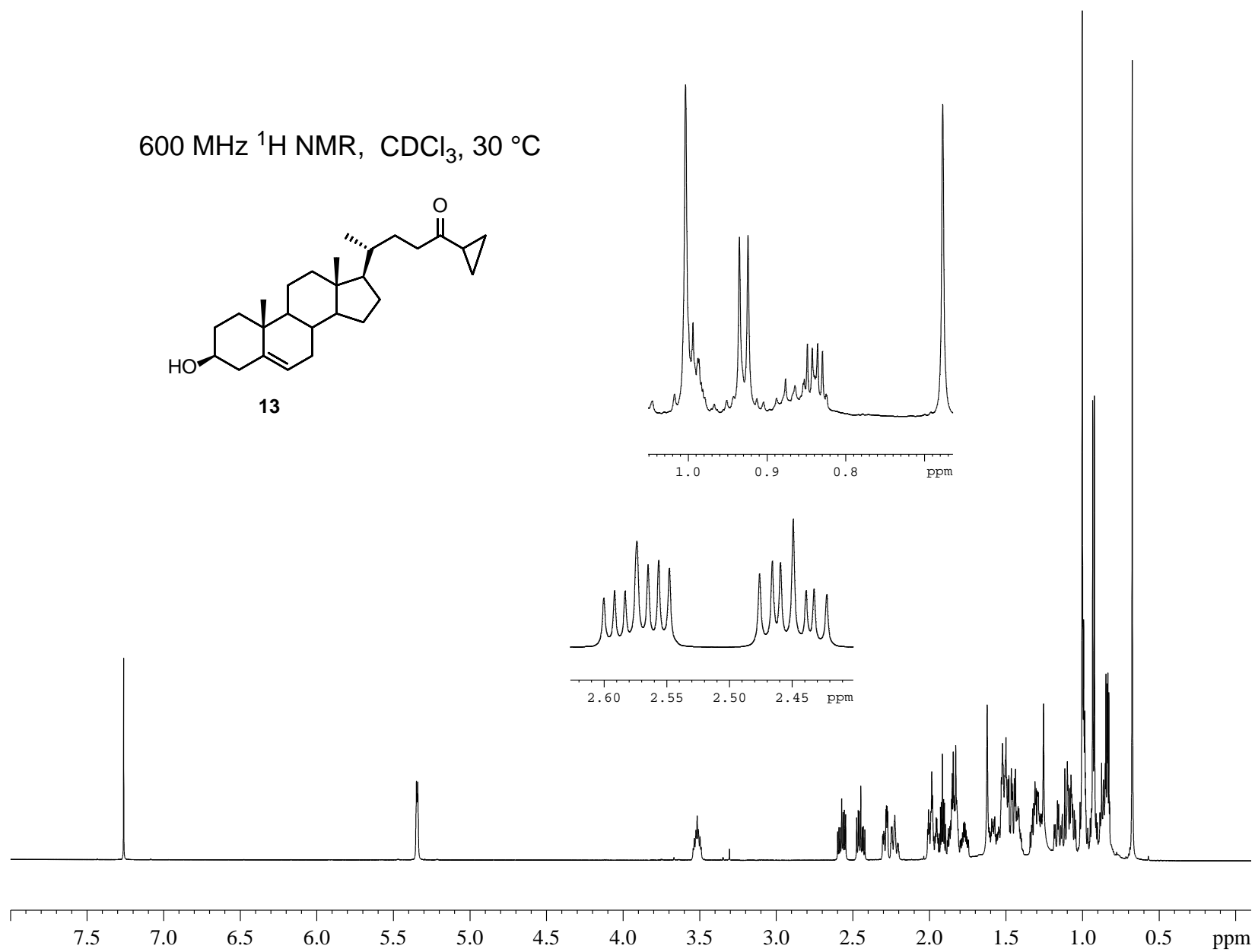
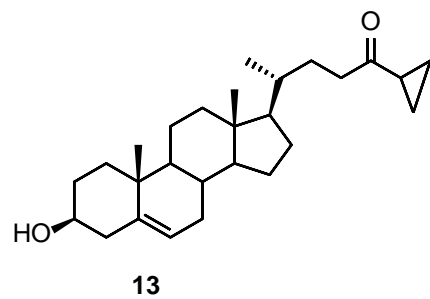
1.0 0.9 0.8 ppm

0.6 0.4 0.2 ppm

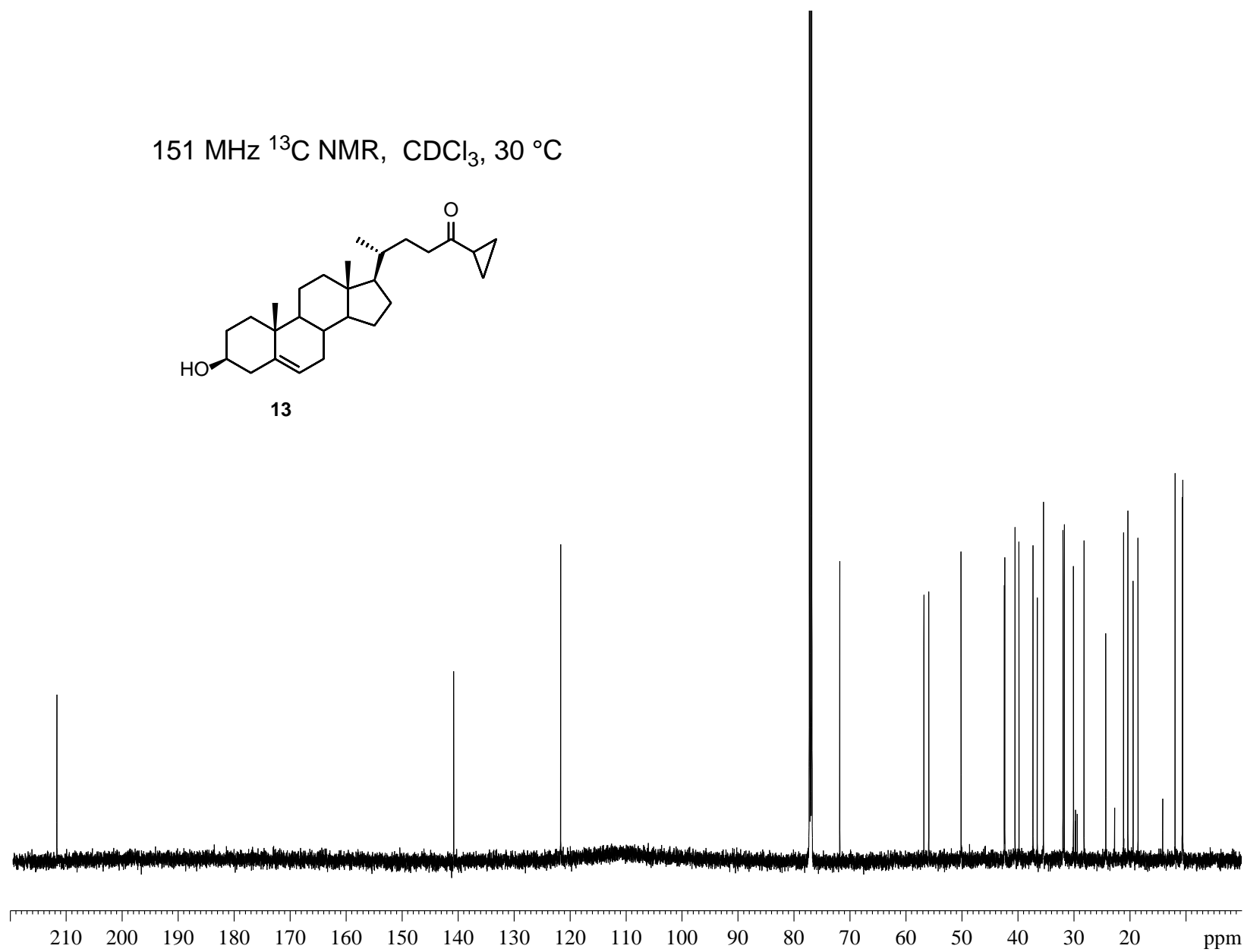
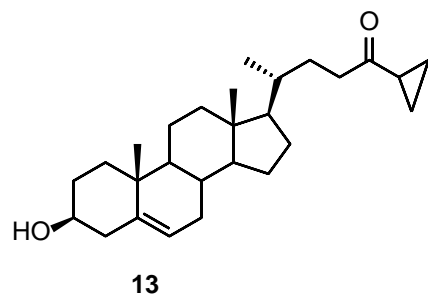
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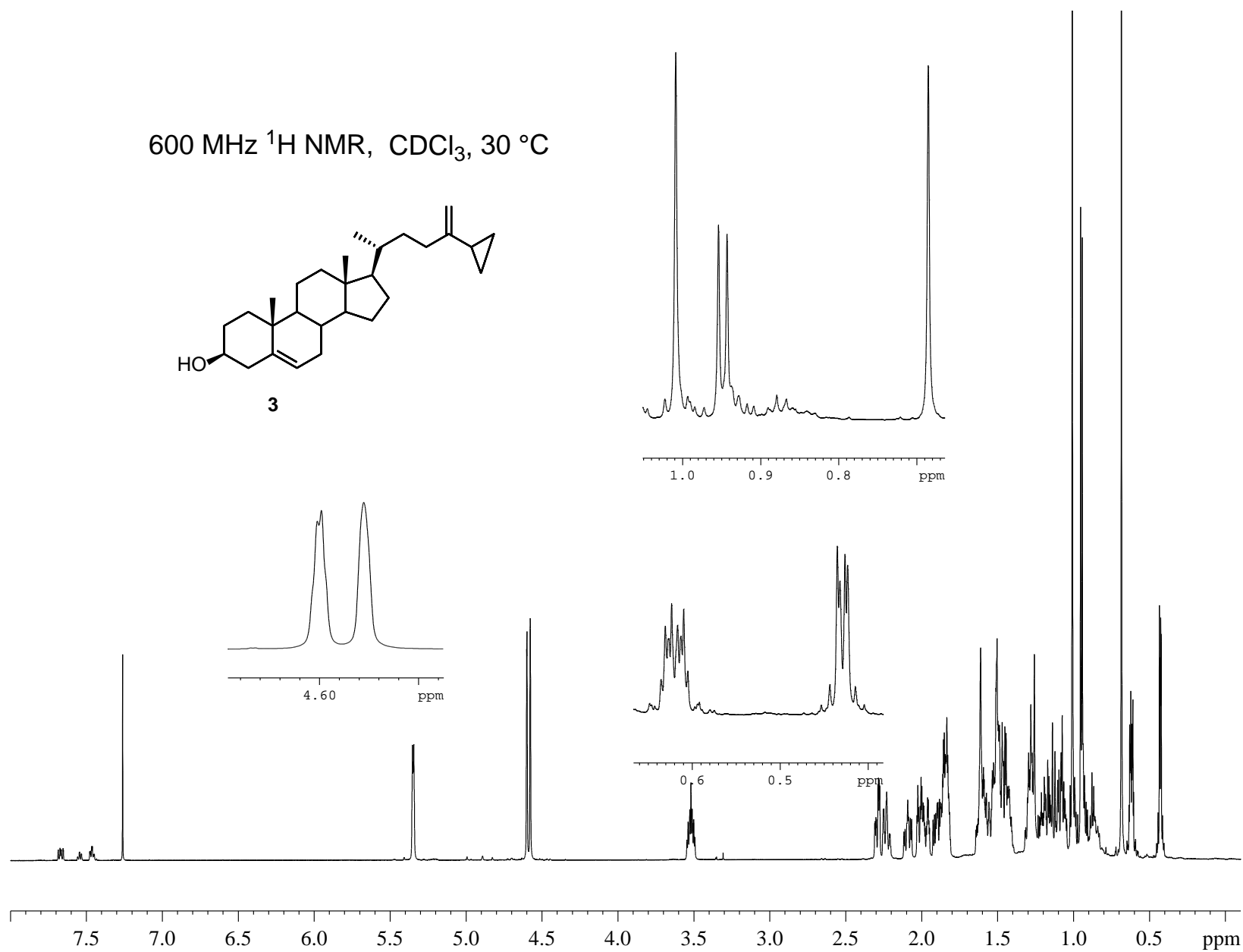
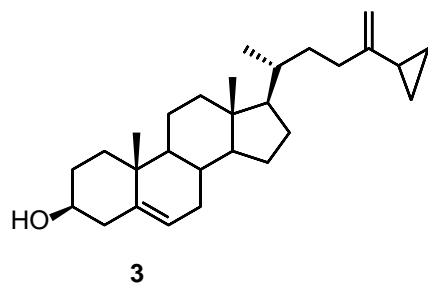
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



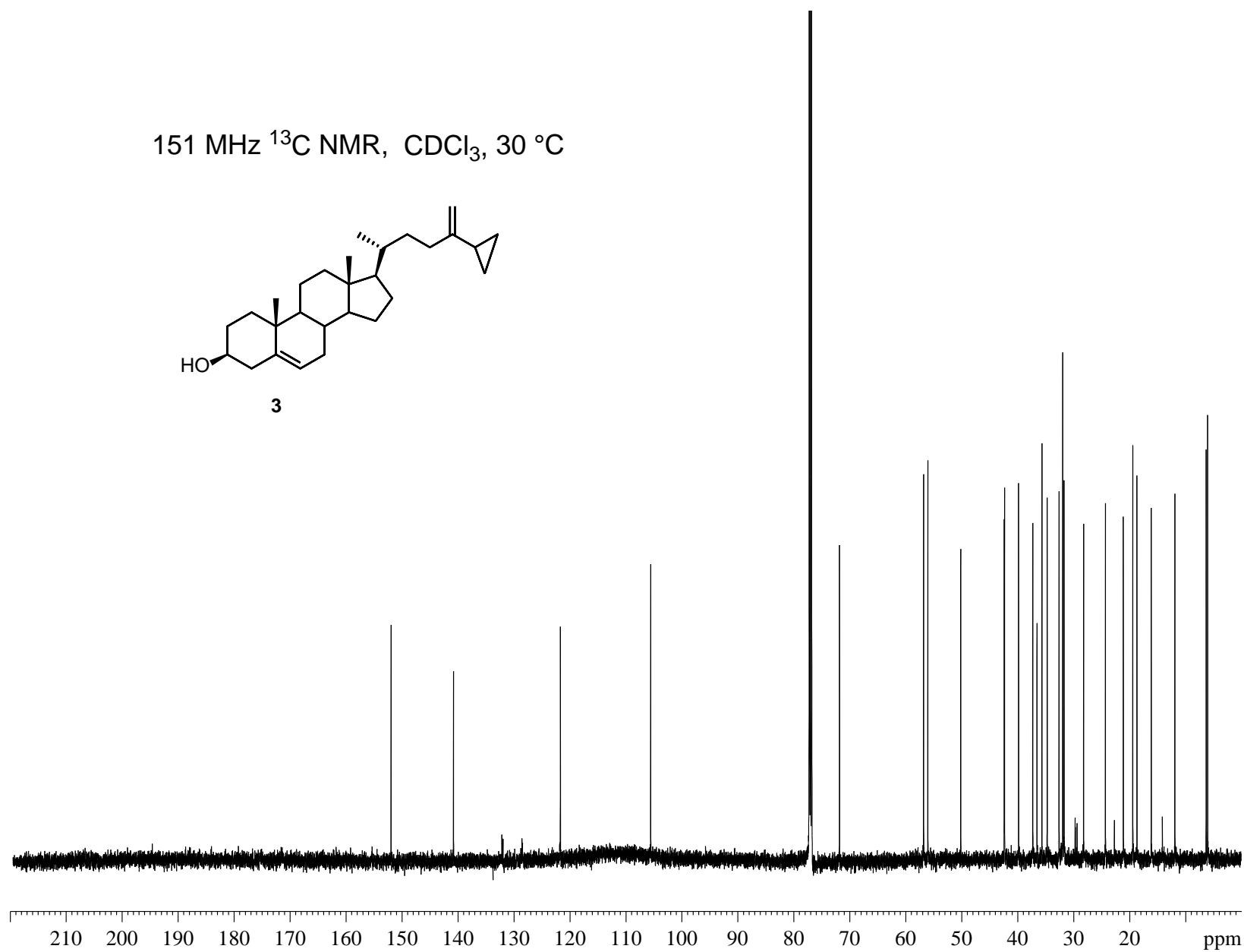
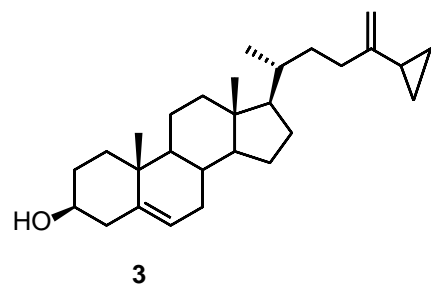
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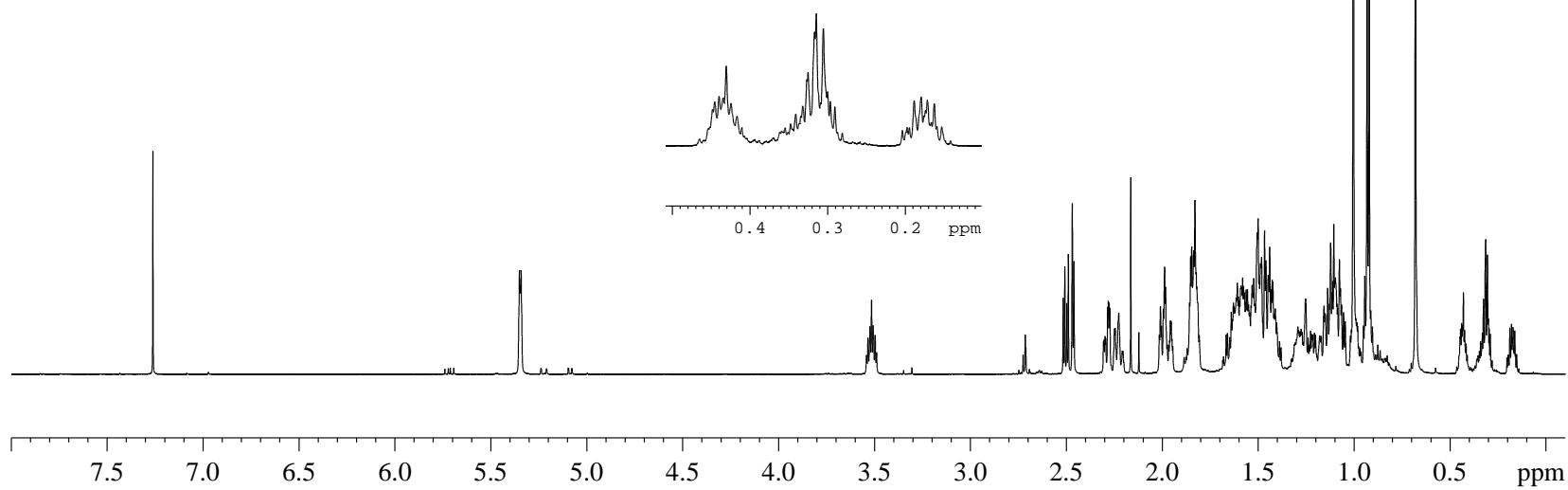
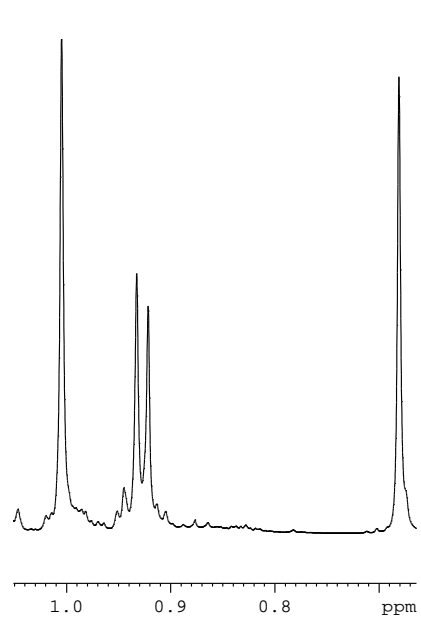
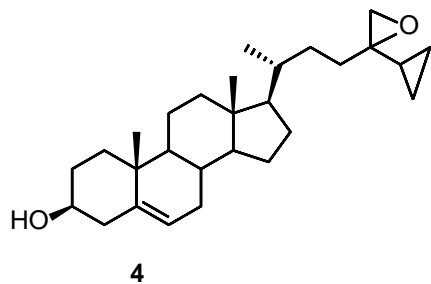
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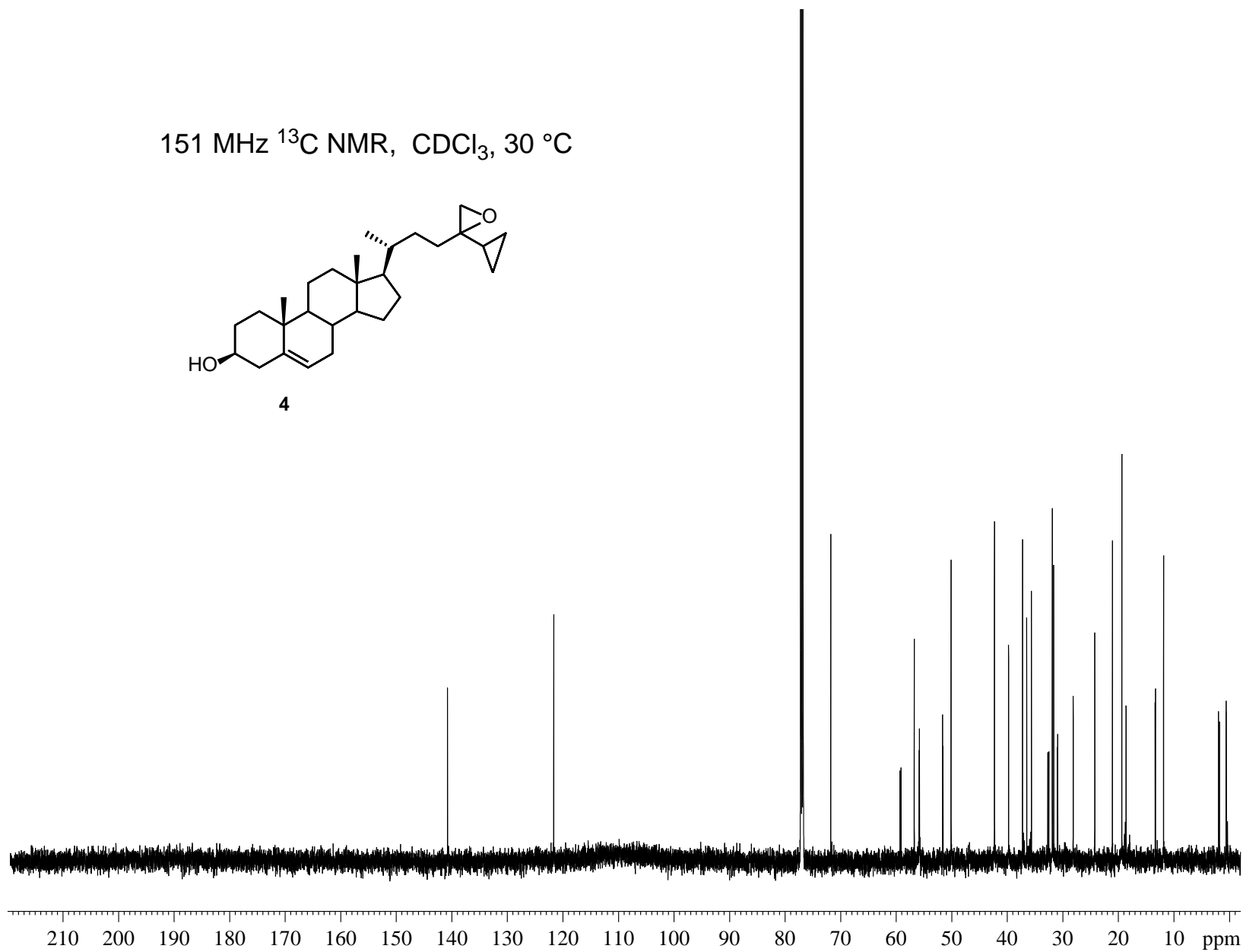
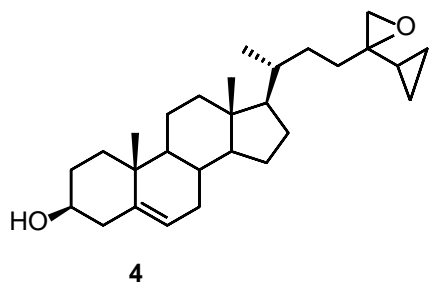
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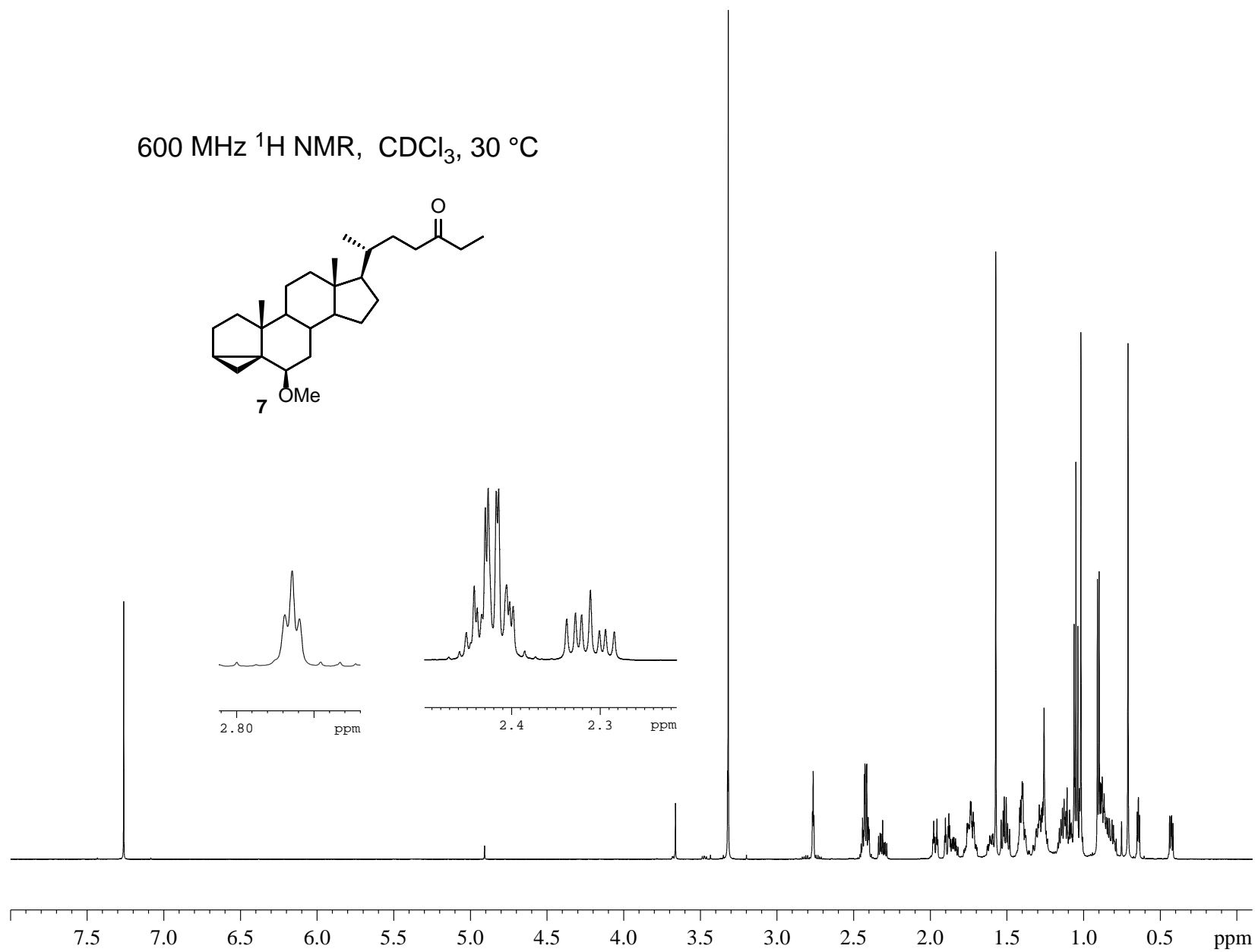
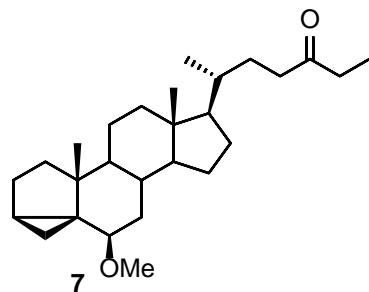
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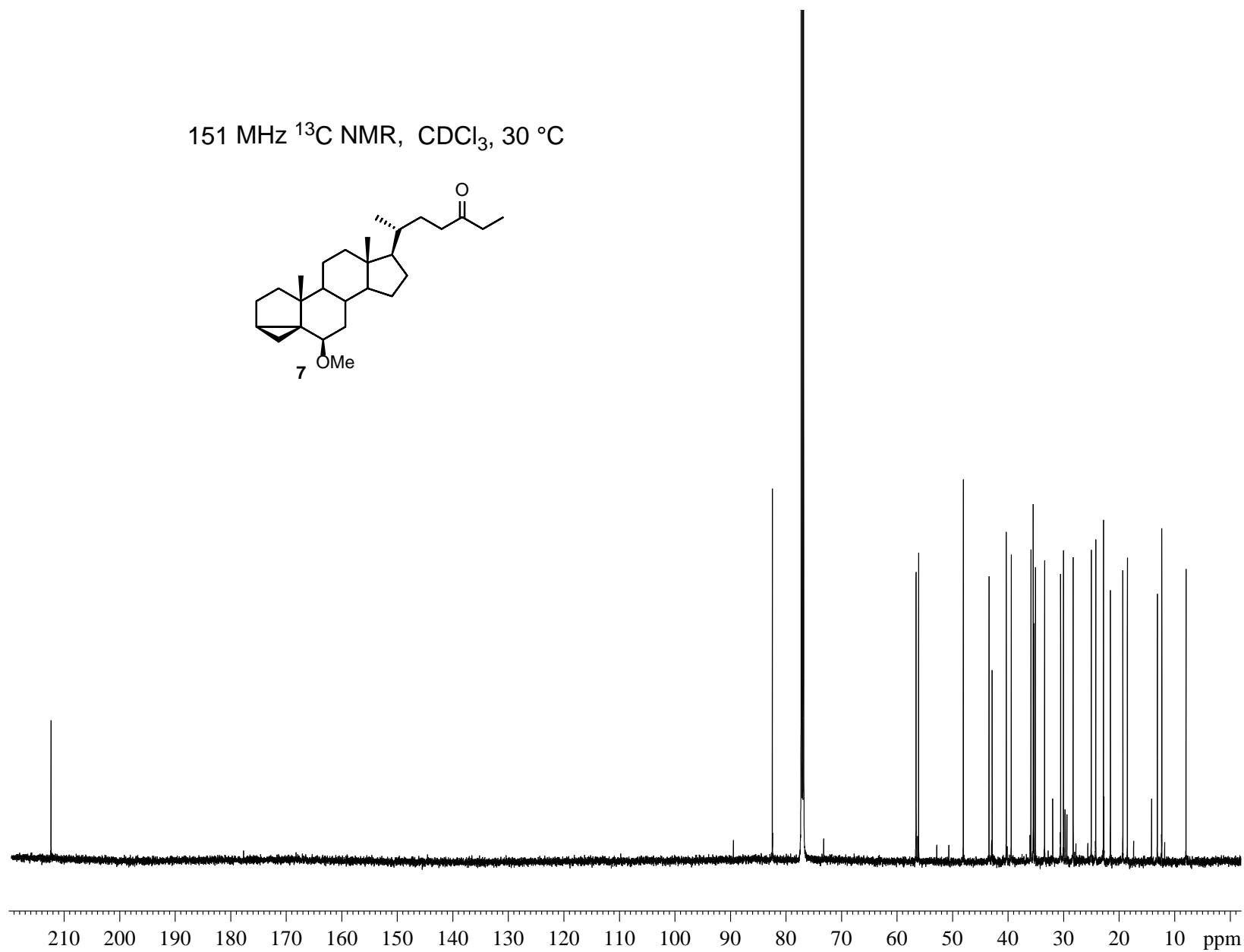
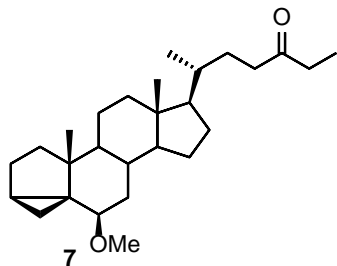
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



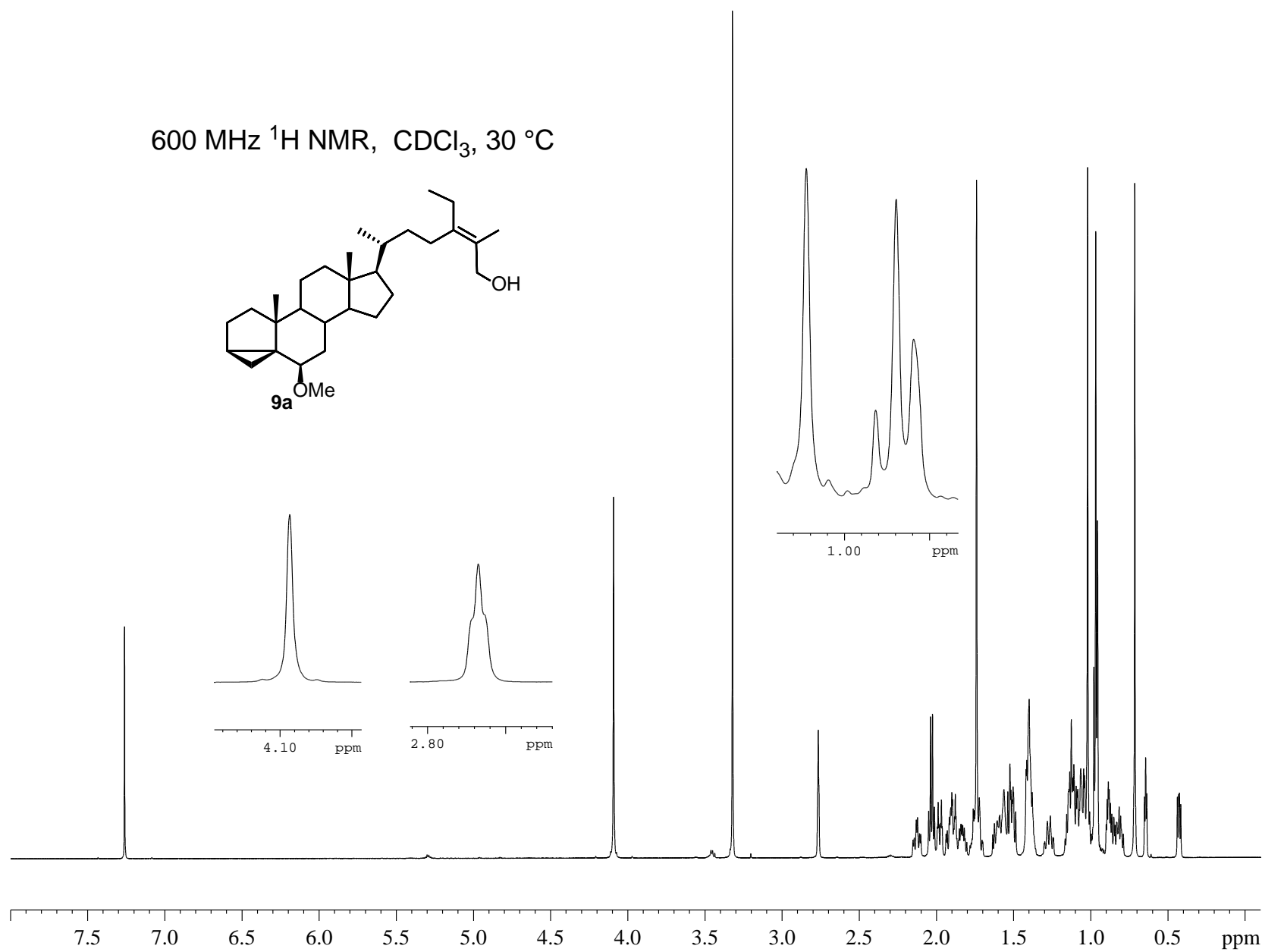
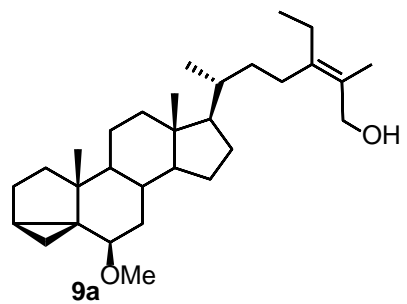
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



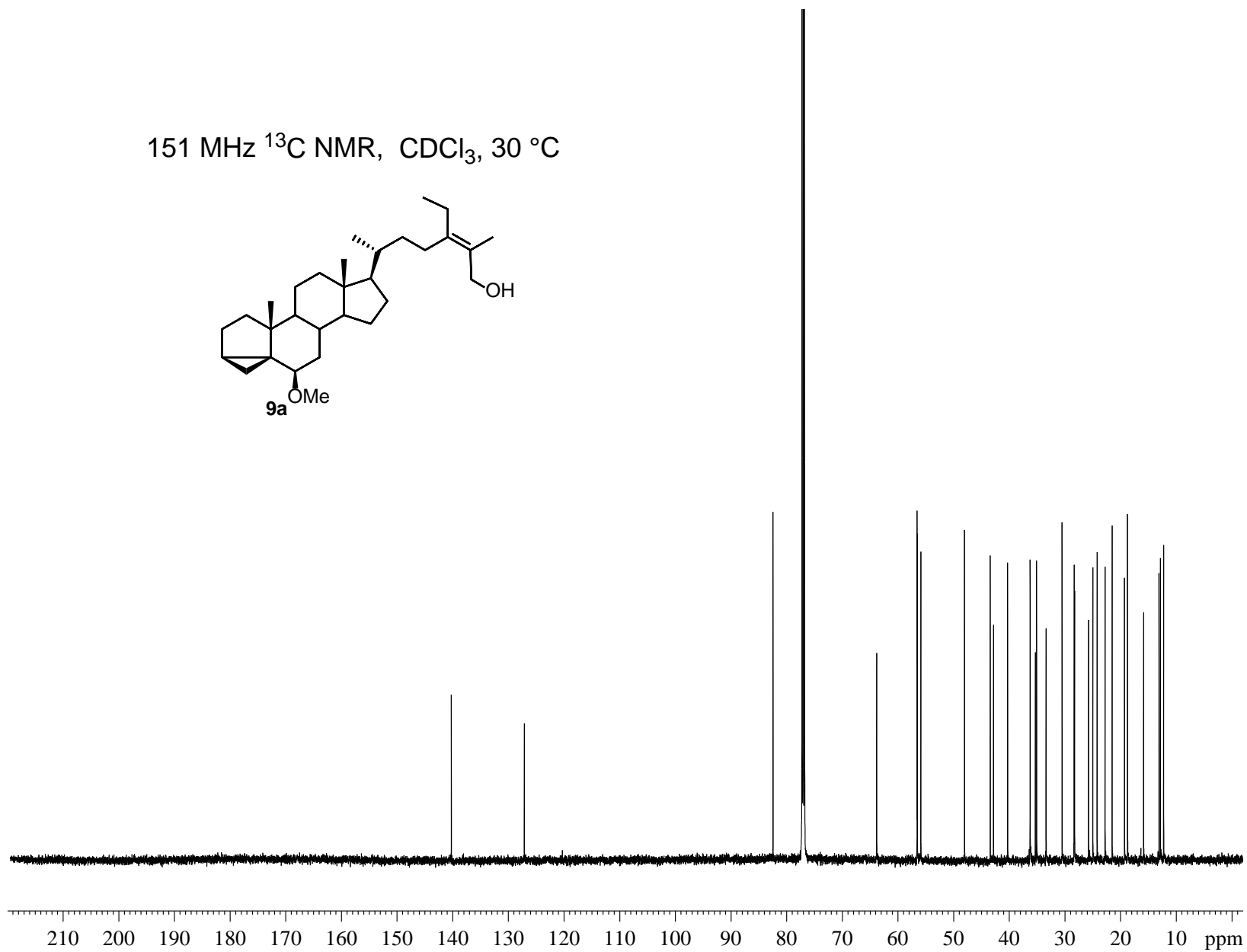
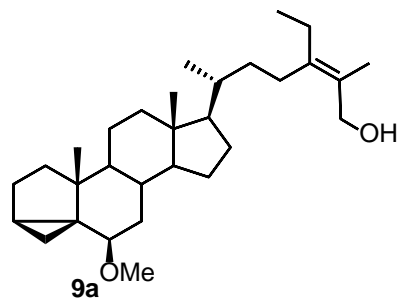
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

9b

Chemical structure of **9b** is shown above the spectrum. The structure is a complex polycyclic molecule with a steroid-like core, featuring a methoxy group (OMe) and a side chain containing a double bond and a hydroxyl group (OH).

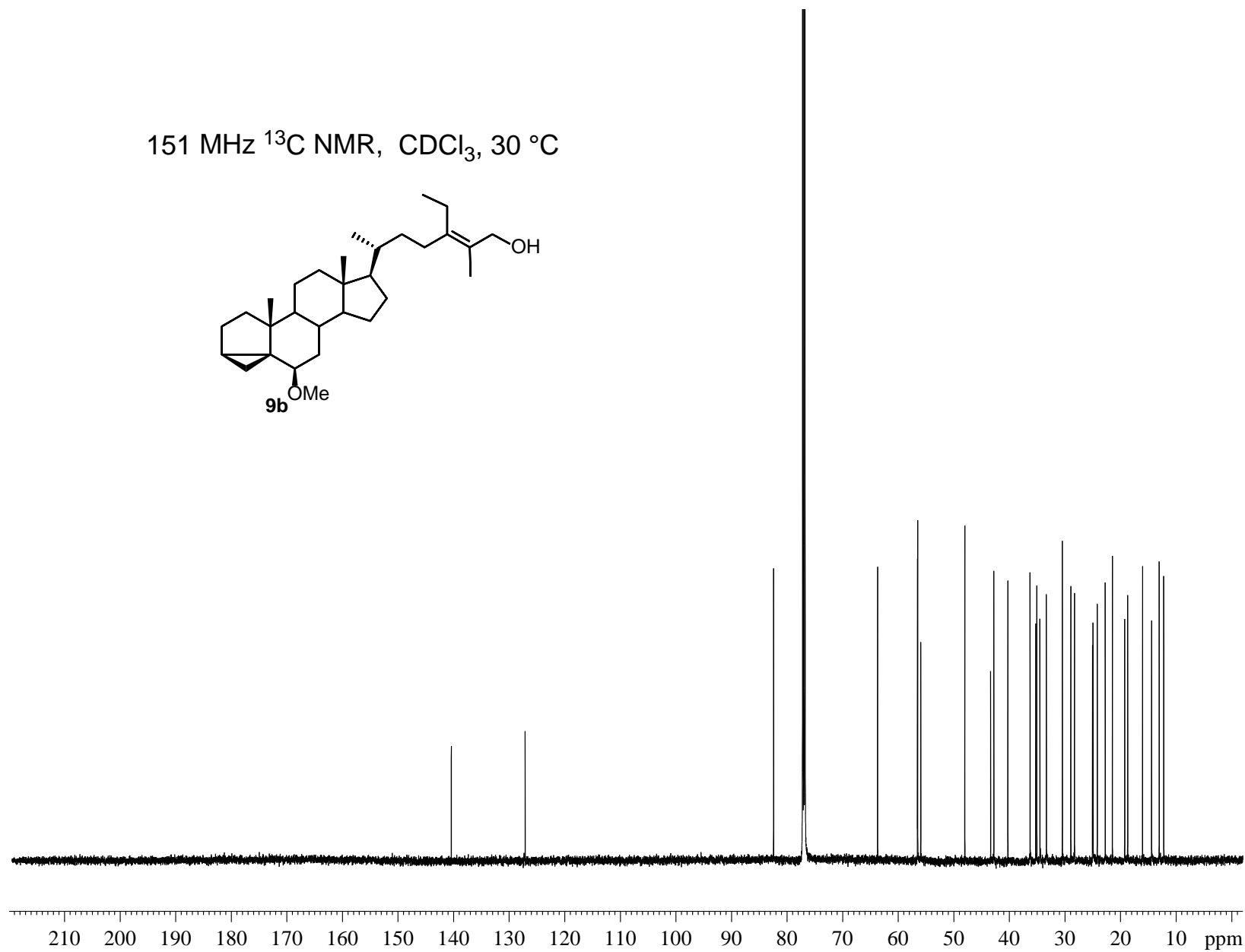
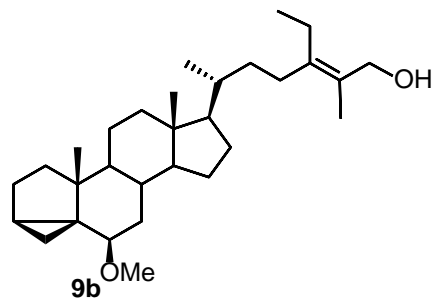
The spectrum displays several peaks, with the following chemical shifts (ppm) indicated by the x-axis:

- ~7.2 ppm (TMS reference peak)
- ~4.1 ppm (doublet, inset)
- ~2.8 ppm (multiplet, inset)
- ~3.5 ppm (broad peak)
- ~2.0 ppm (broad peak)
- ~1.0 ppm (broad peak)
- ~0.5 ppm (broad peak)

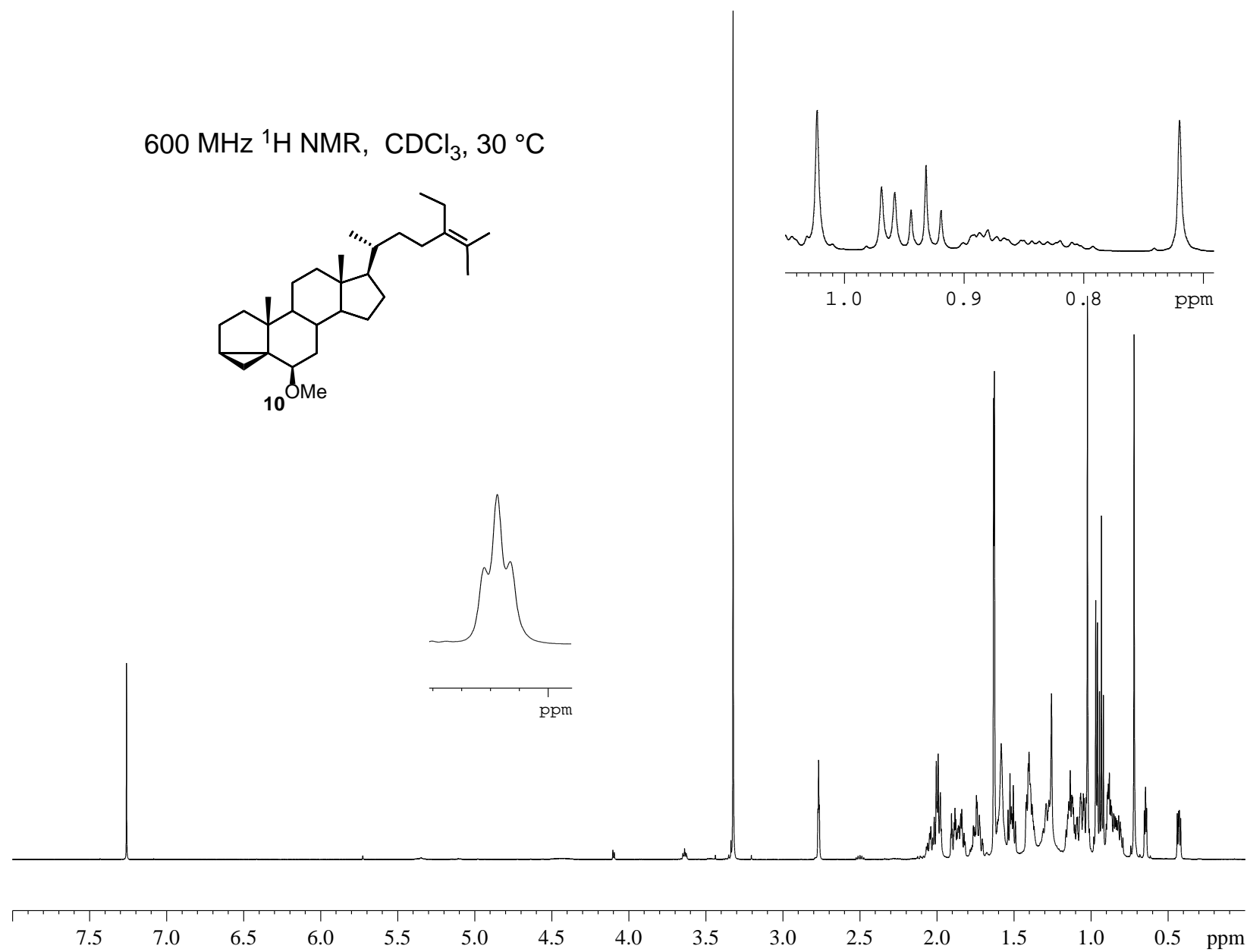
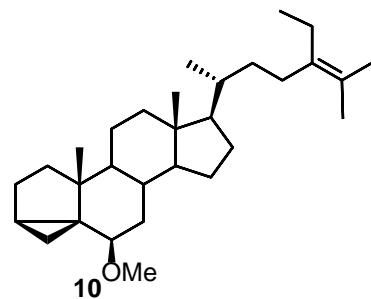
Integration values are provided for the peaks:

- 1.00 (for the peak at ~4.1 ppm)
- 1.00 (for the peak at ~2.8 ppm)

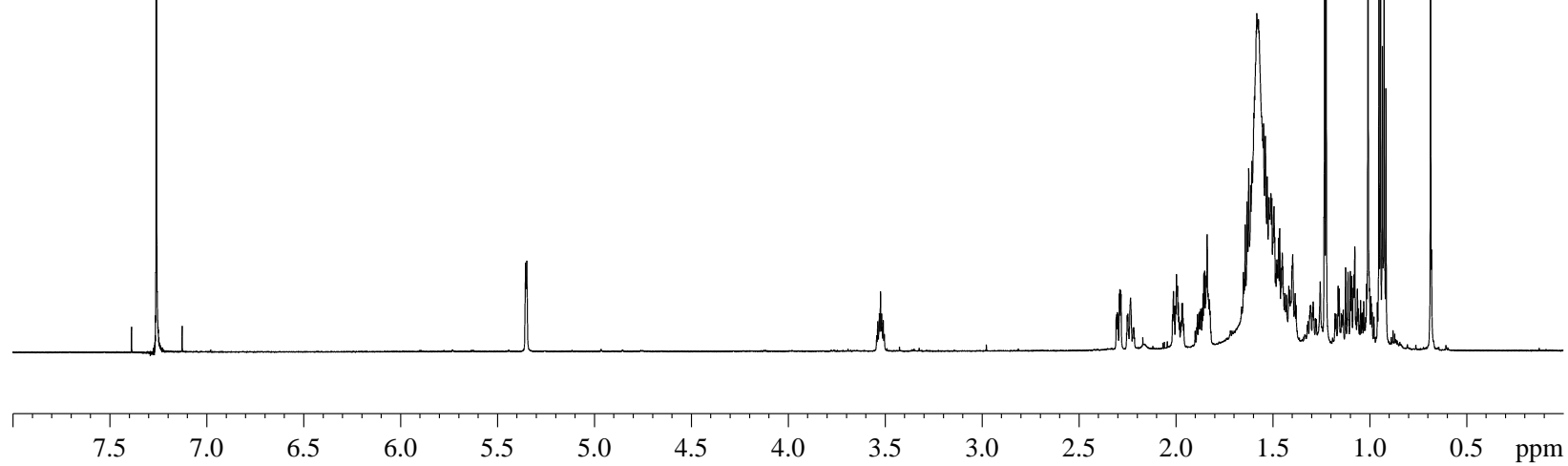
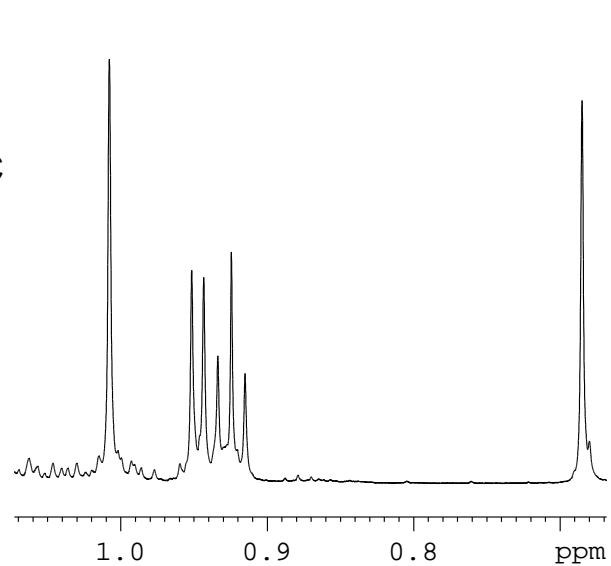
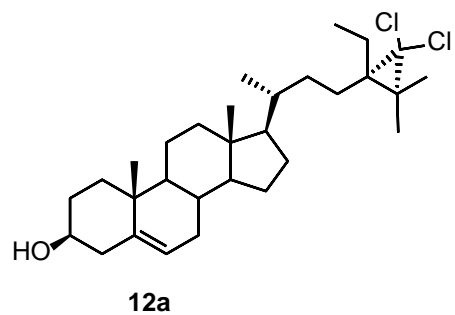
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$

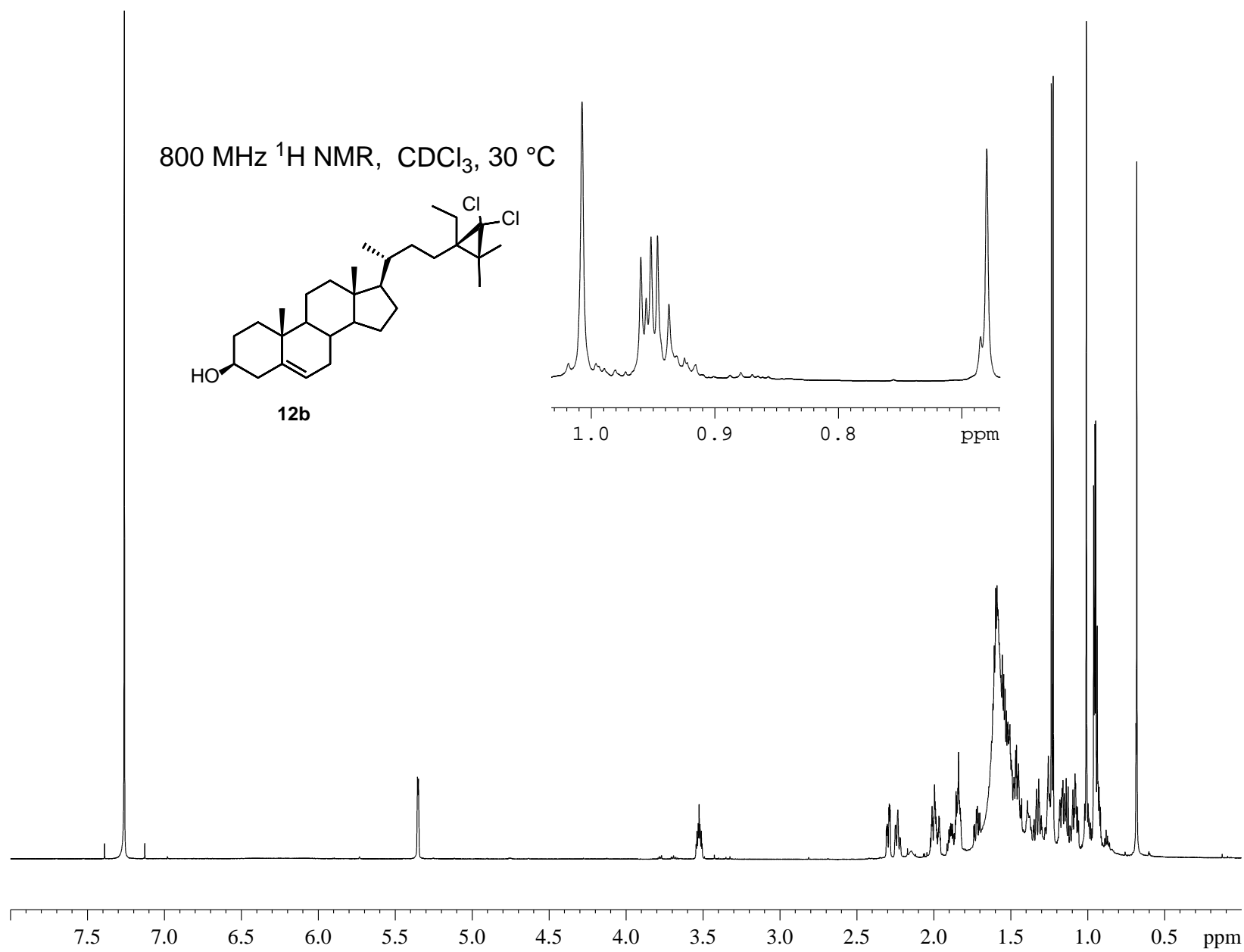


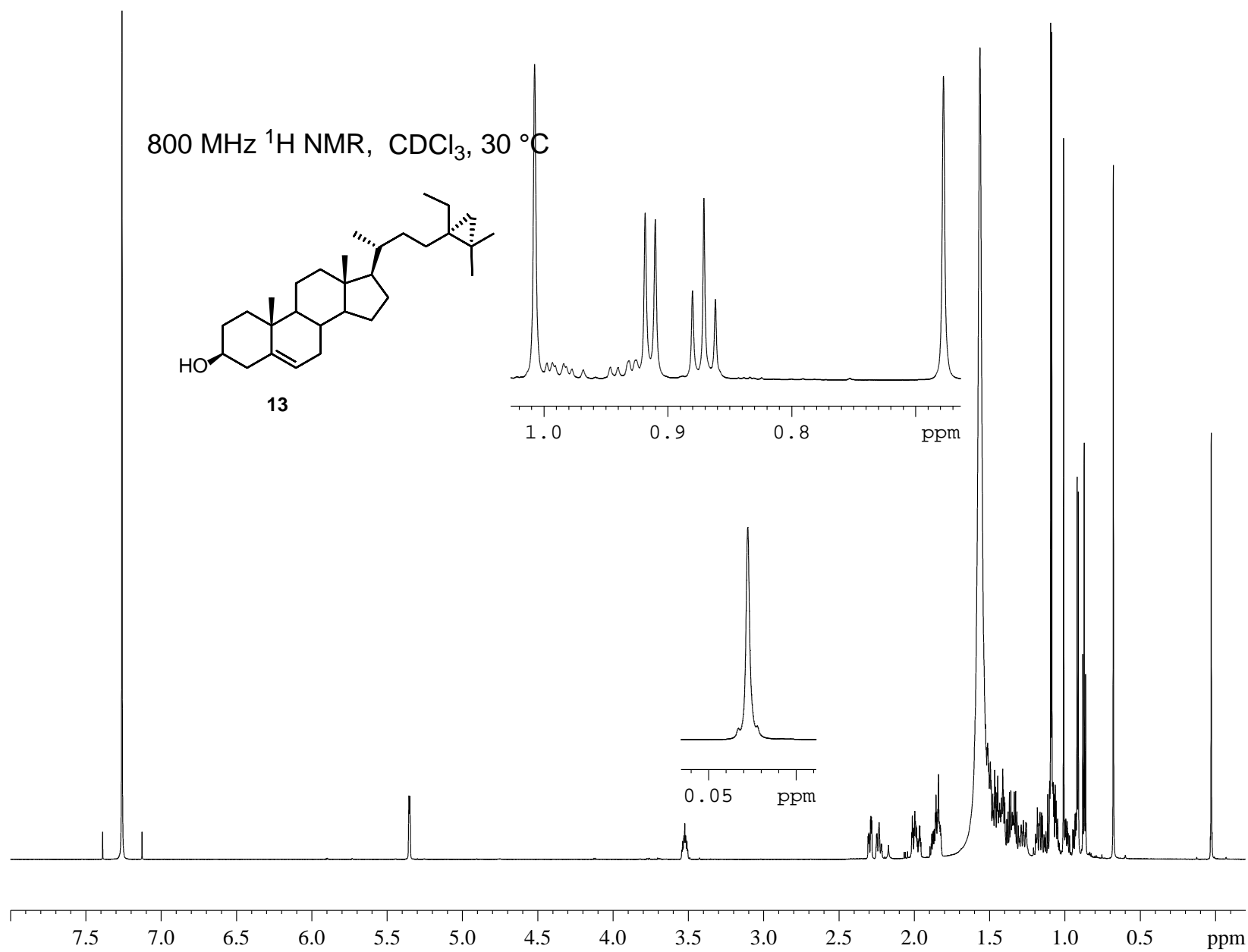
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

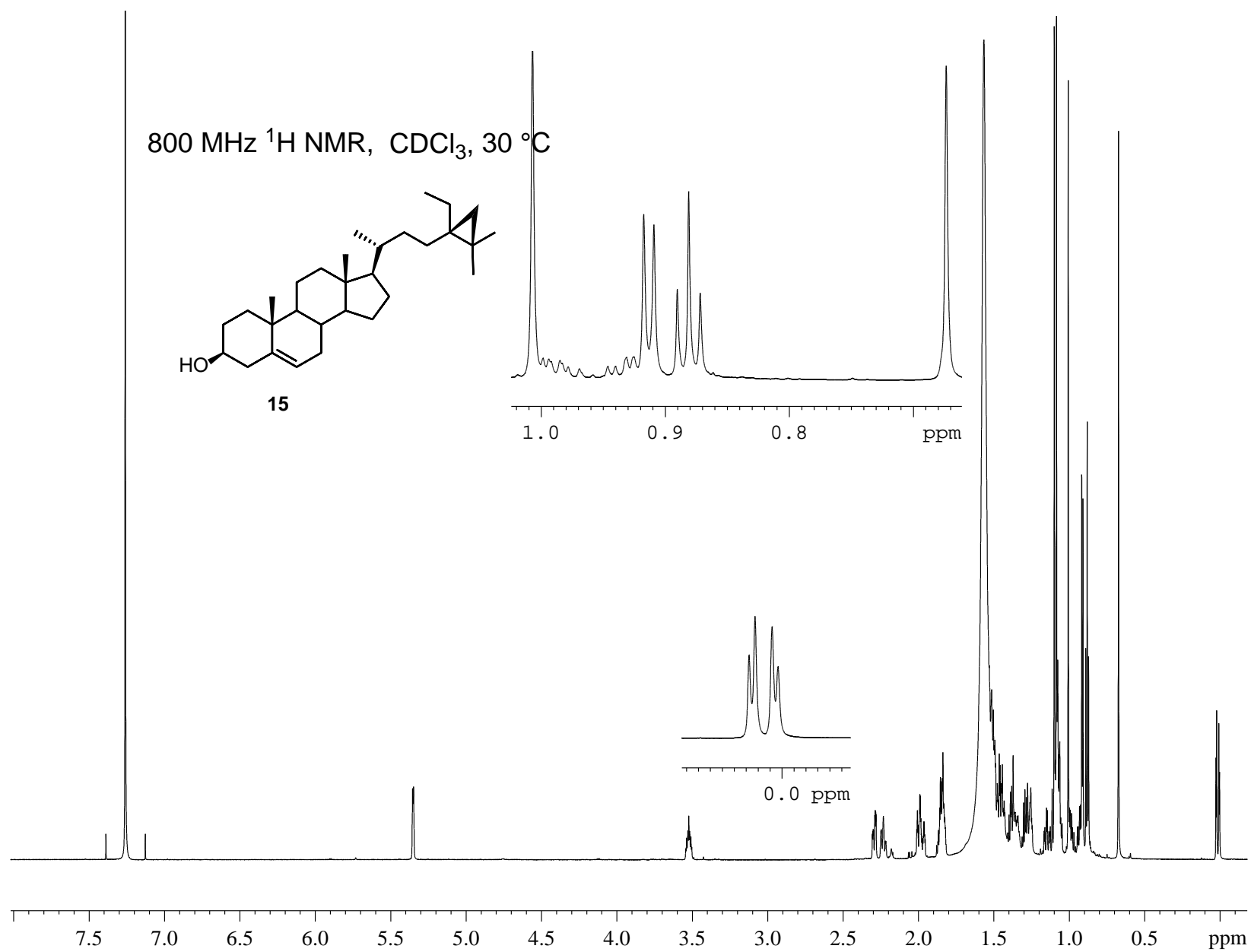


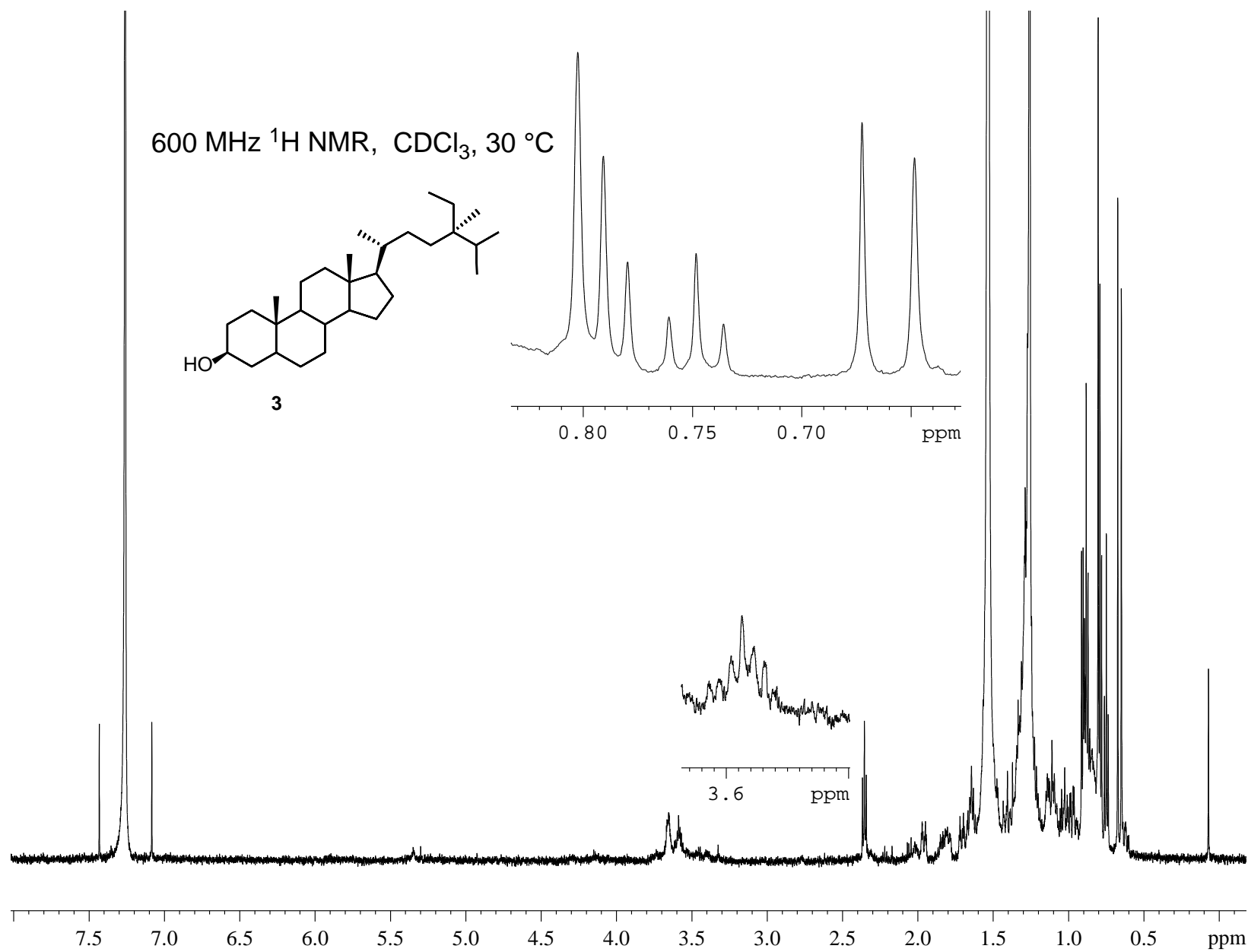
800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

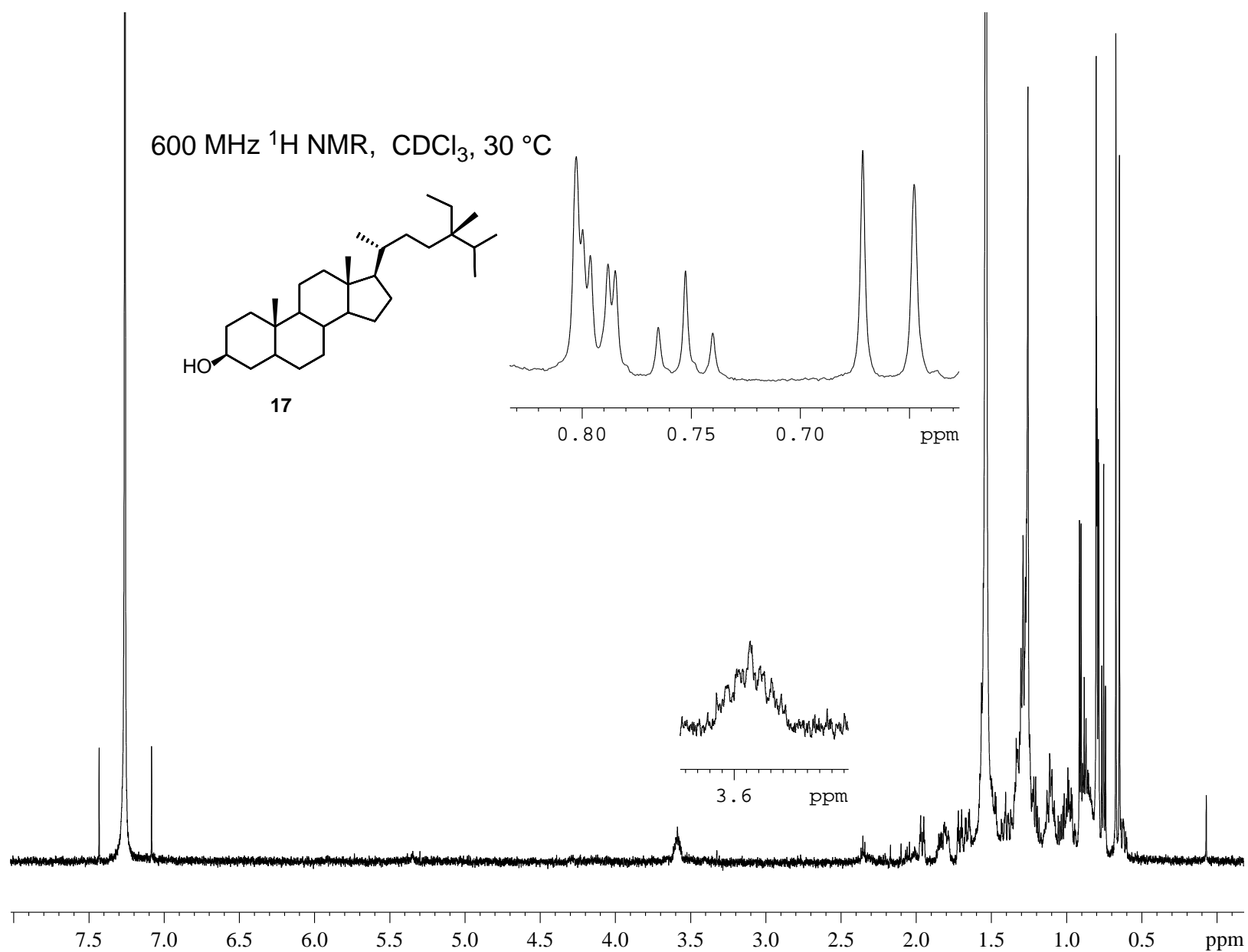


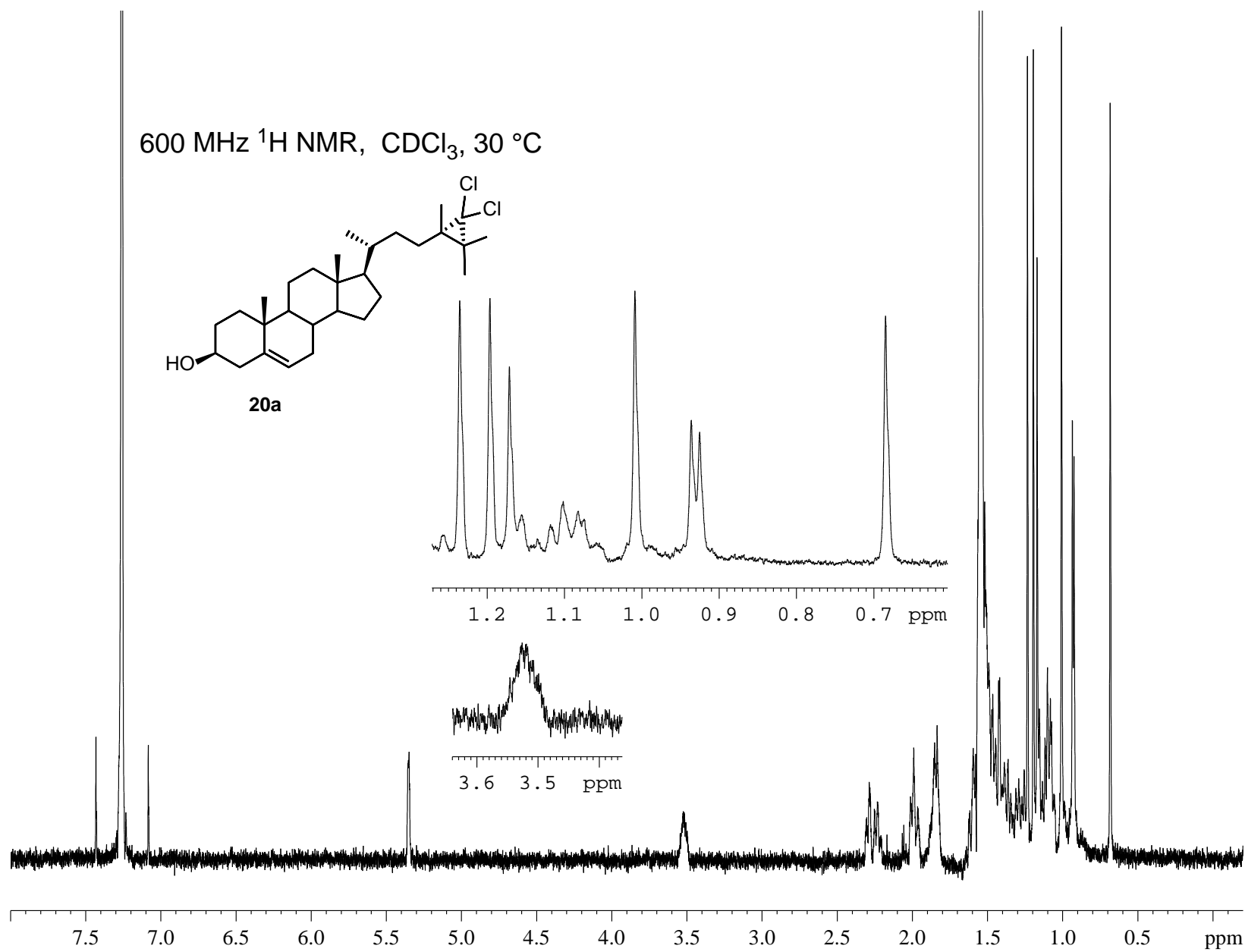


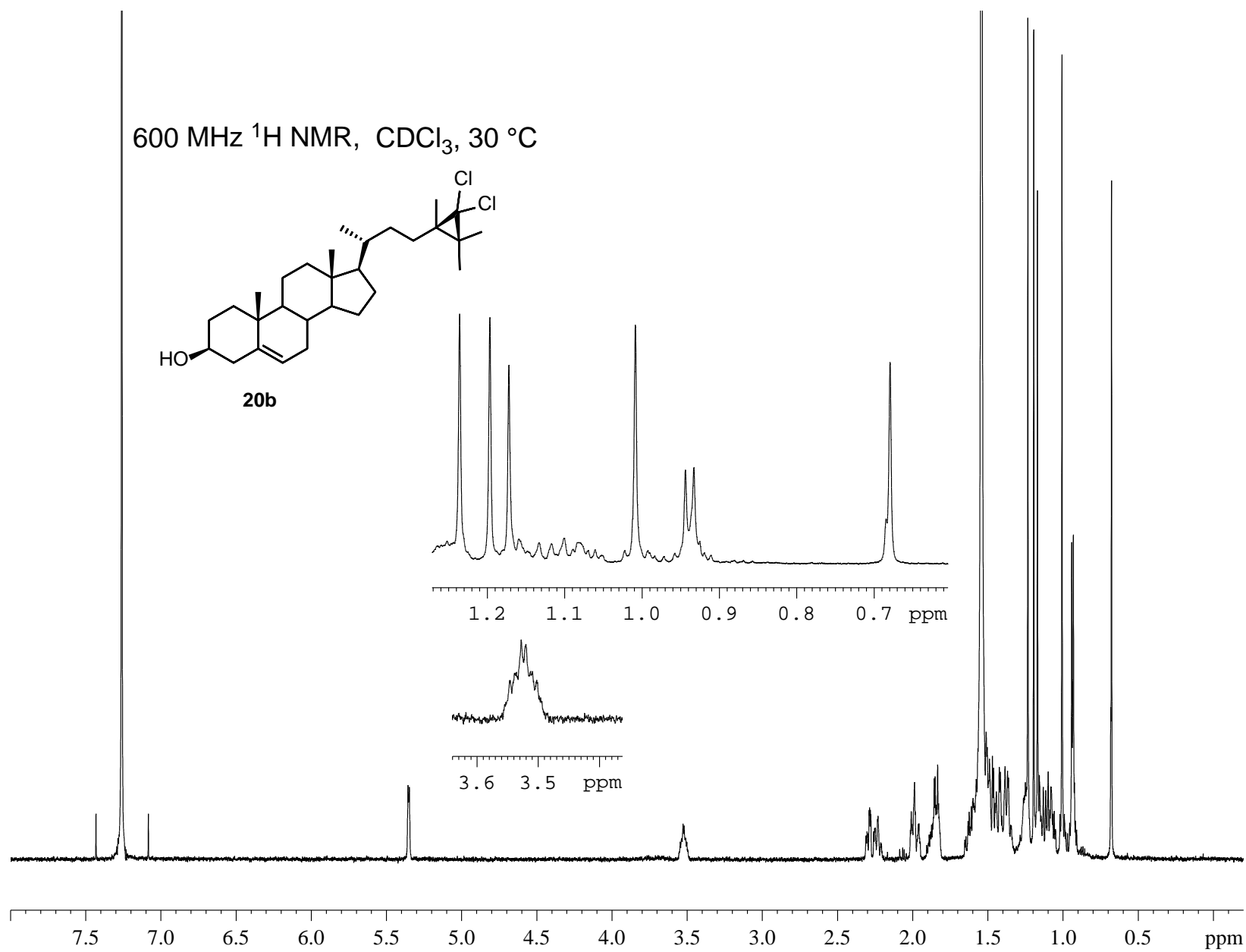




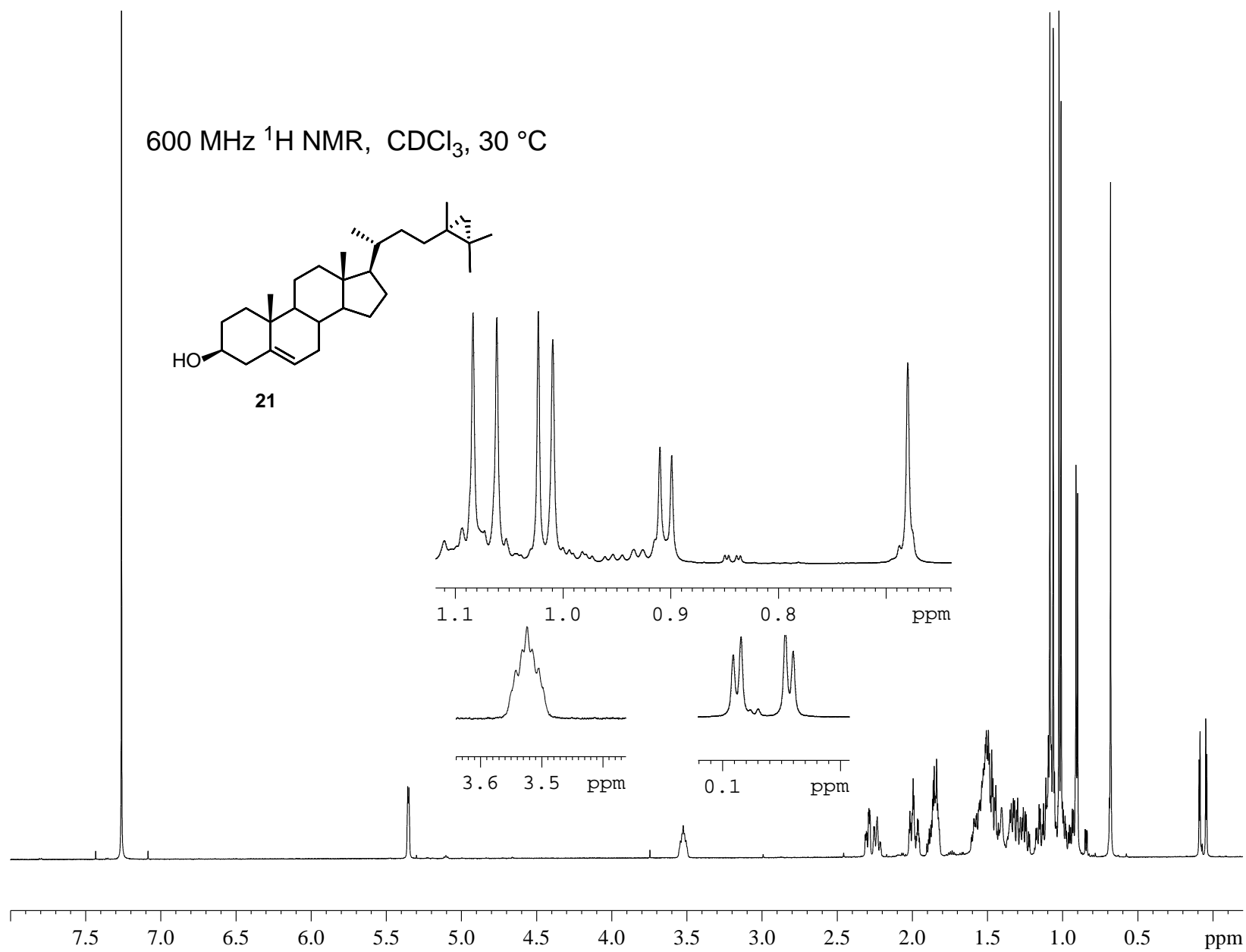
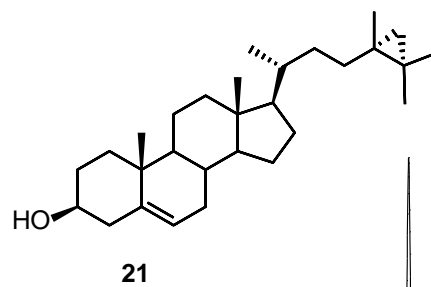




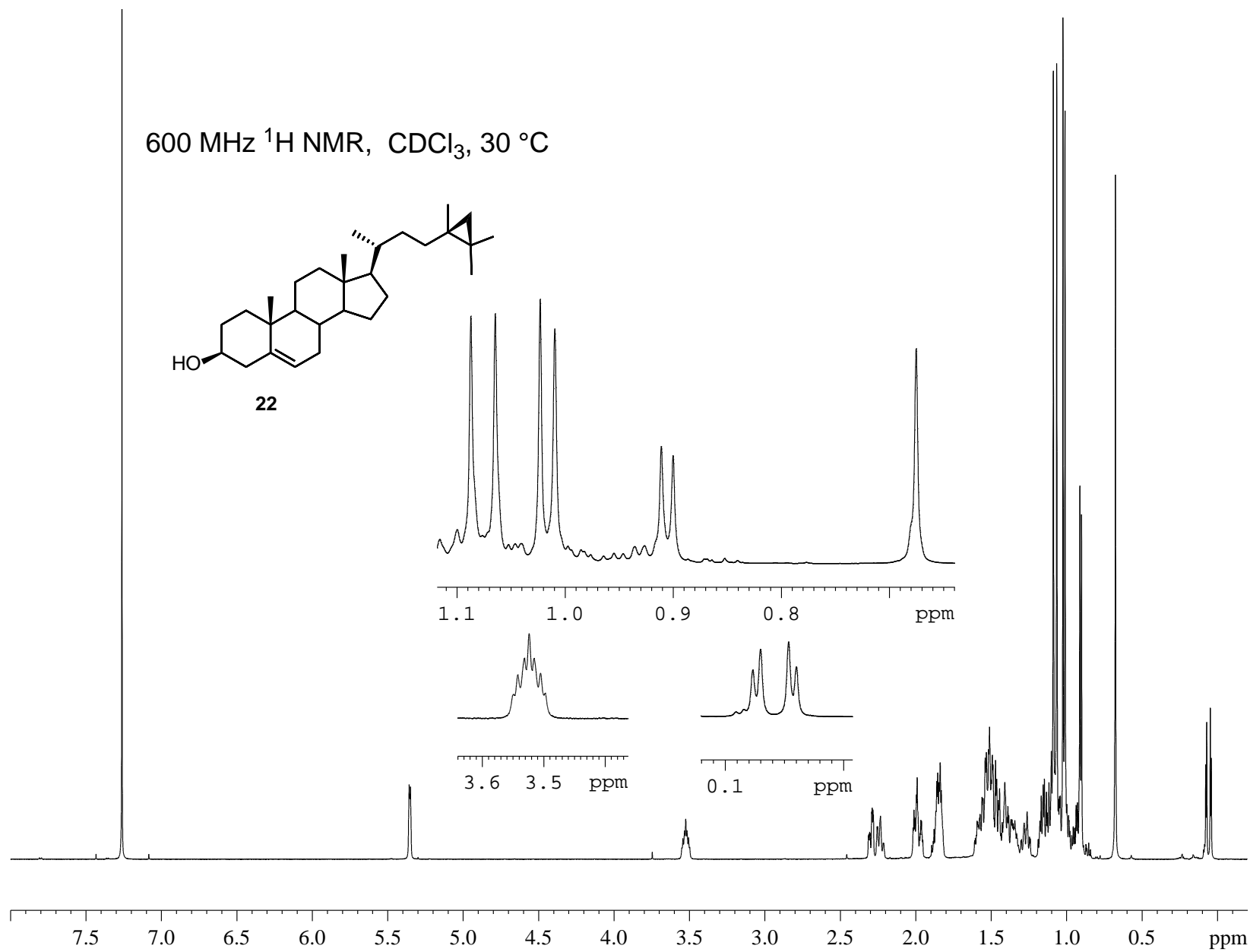
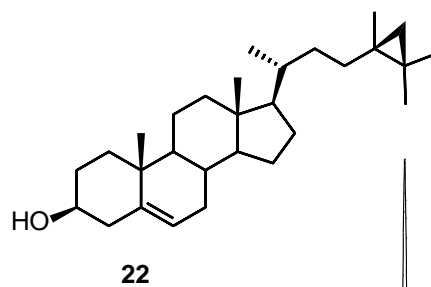


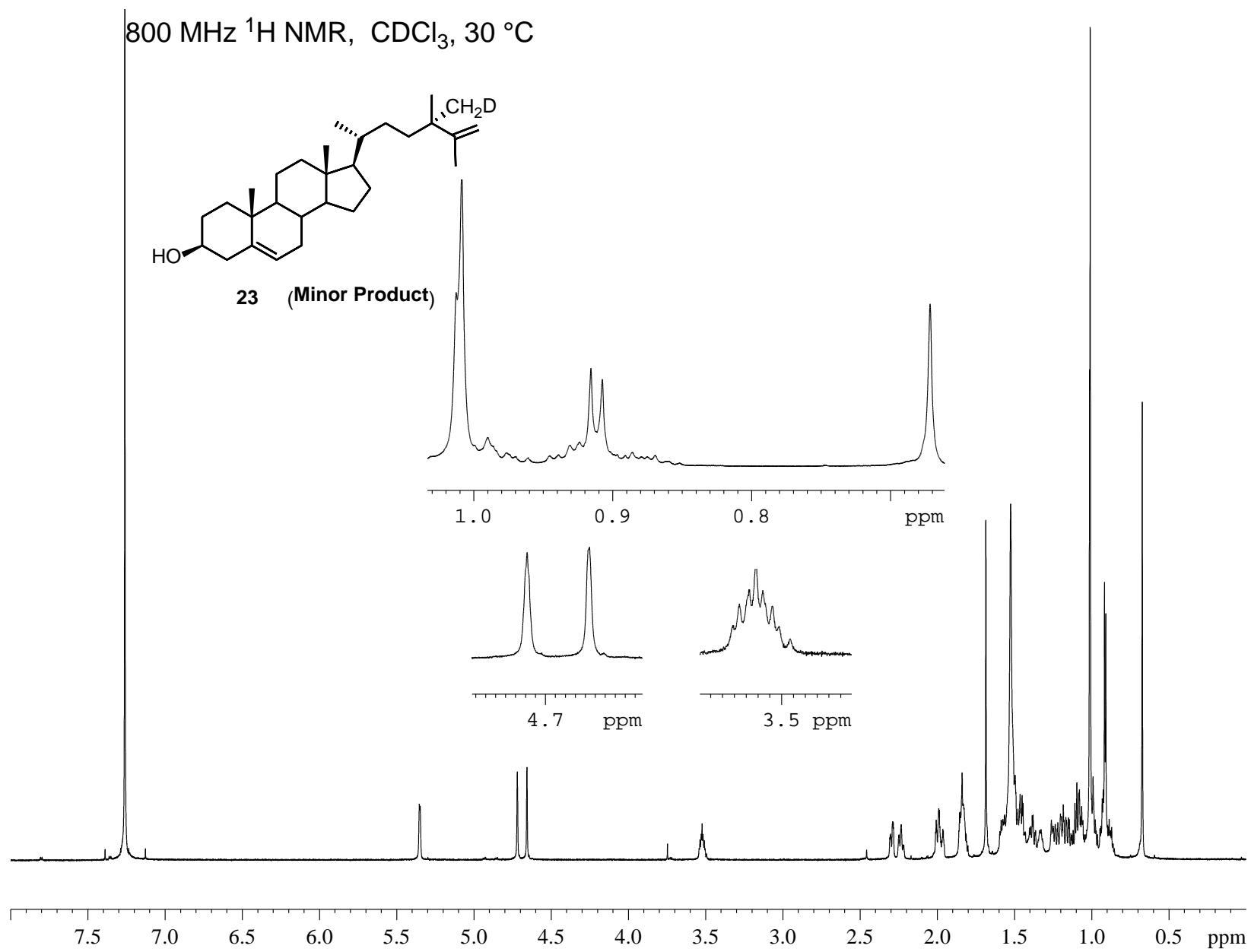


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

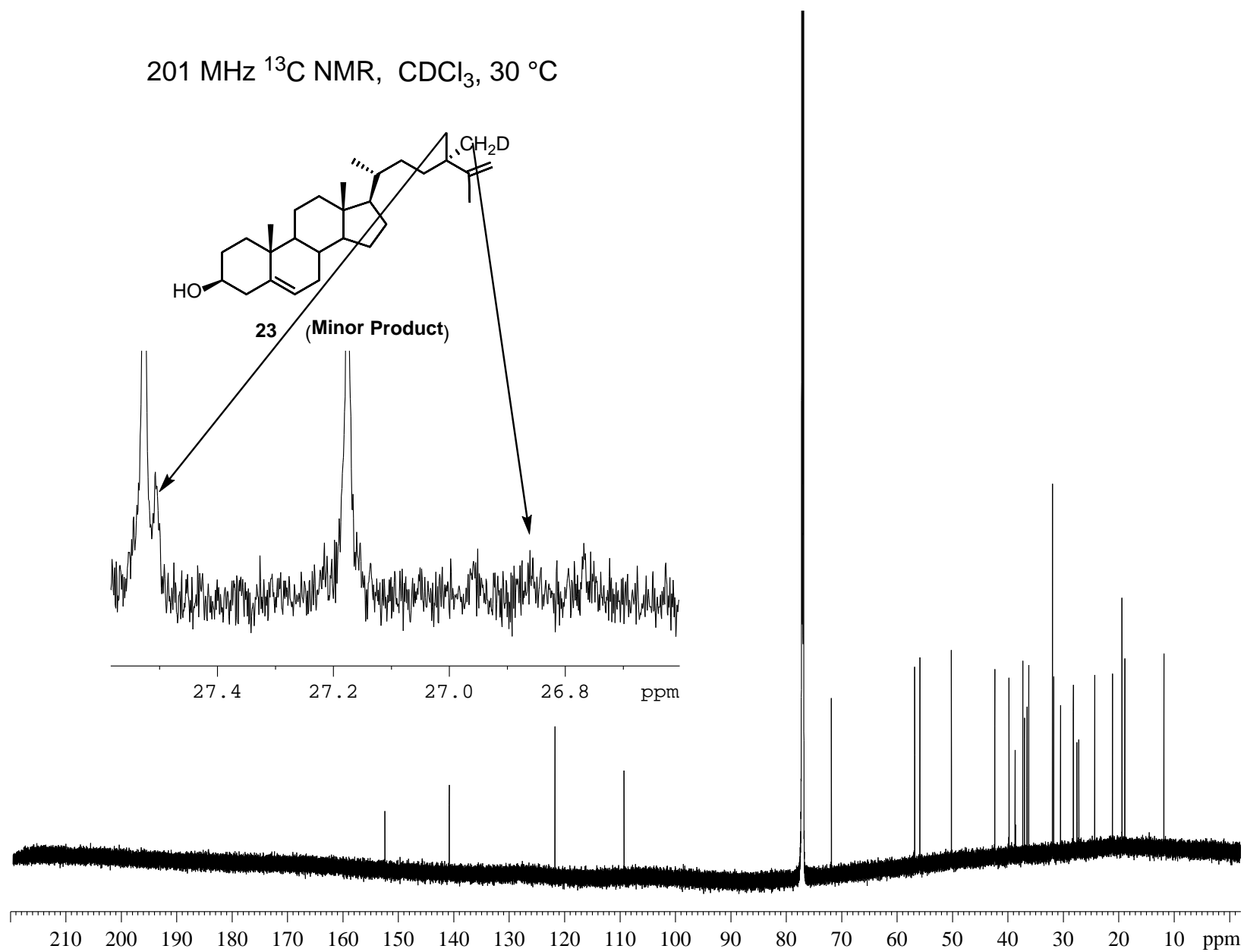


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

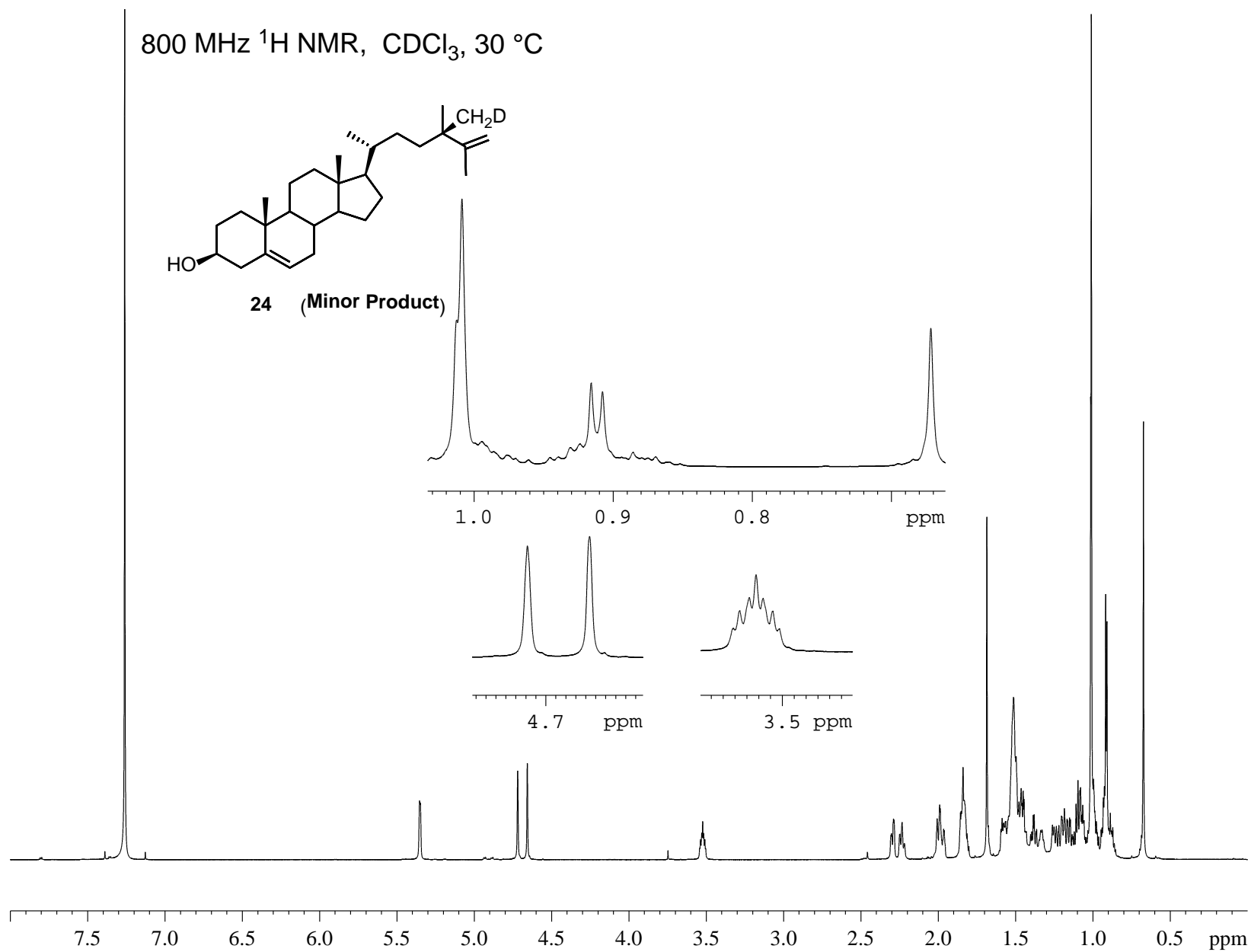
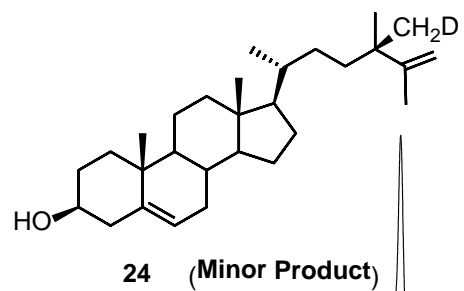




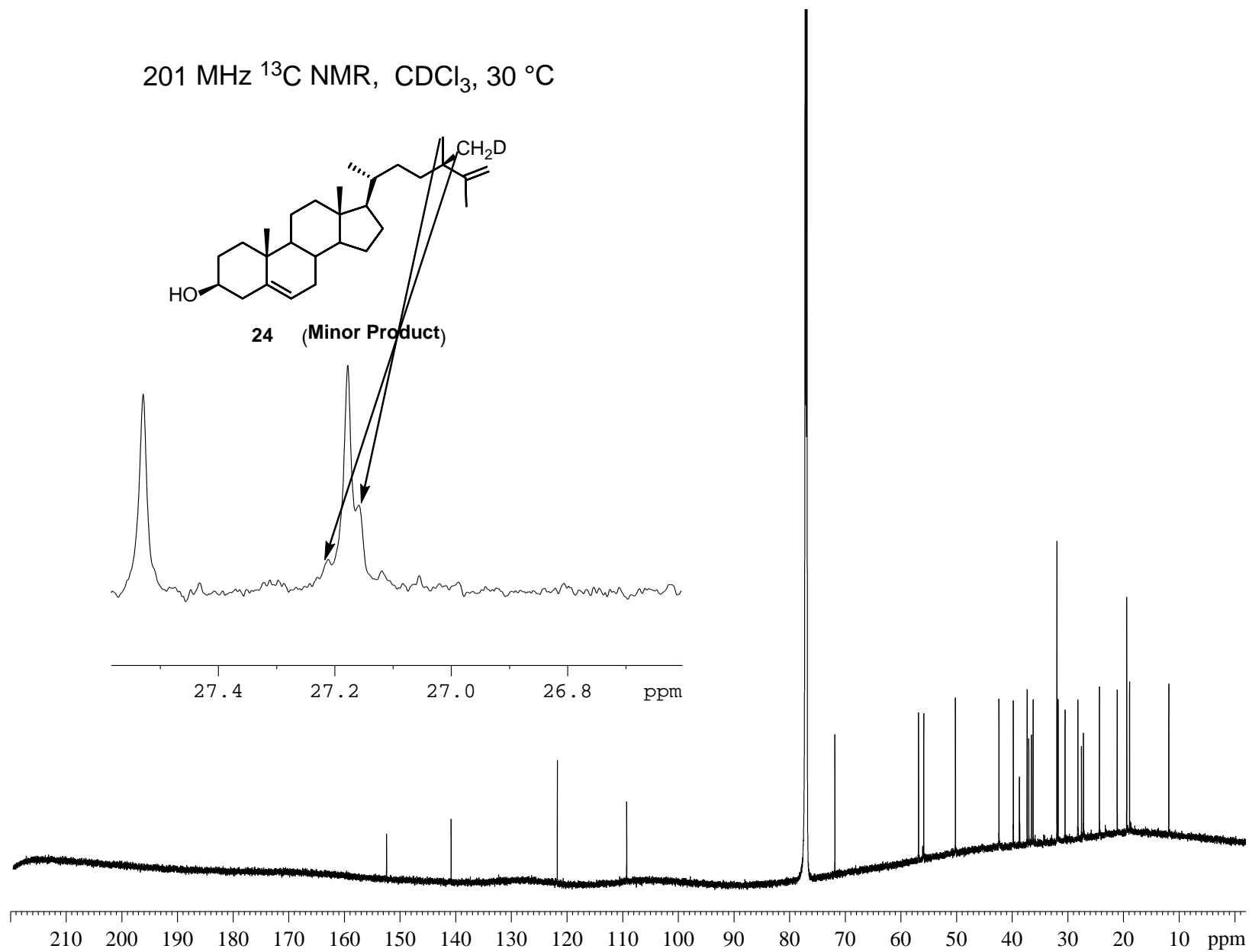
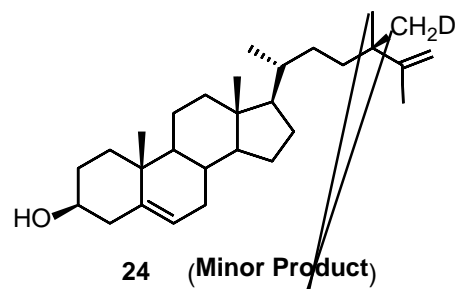
201 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



201 MHz ^{13}C NMR, CDCl_3 , 30 $^{\circ}\text{C}$



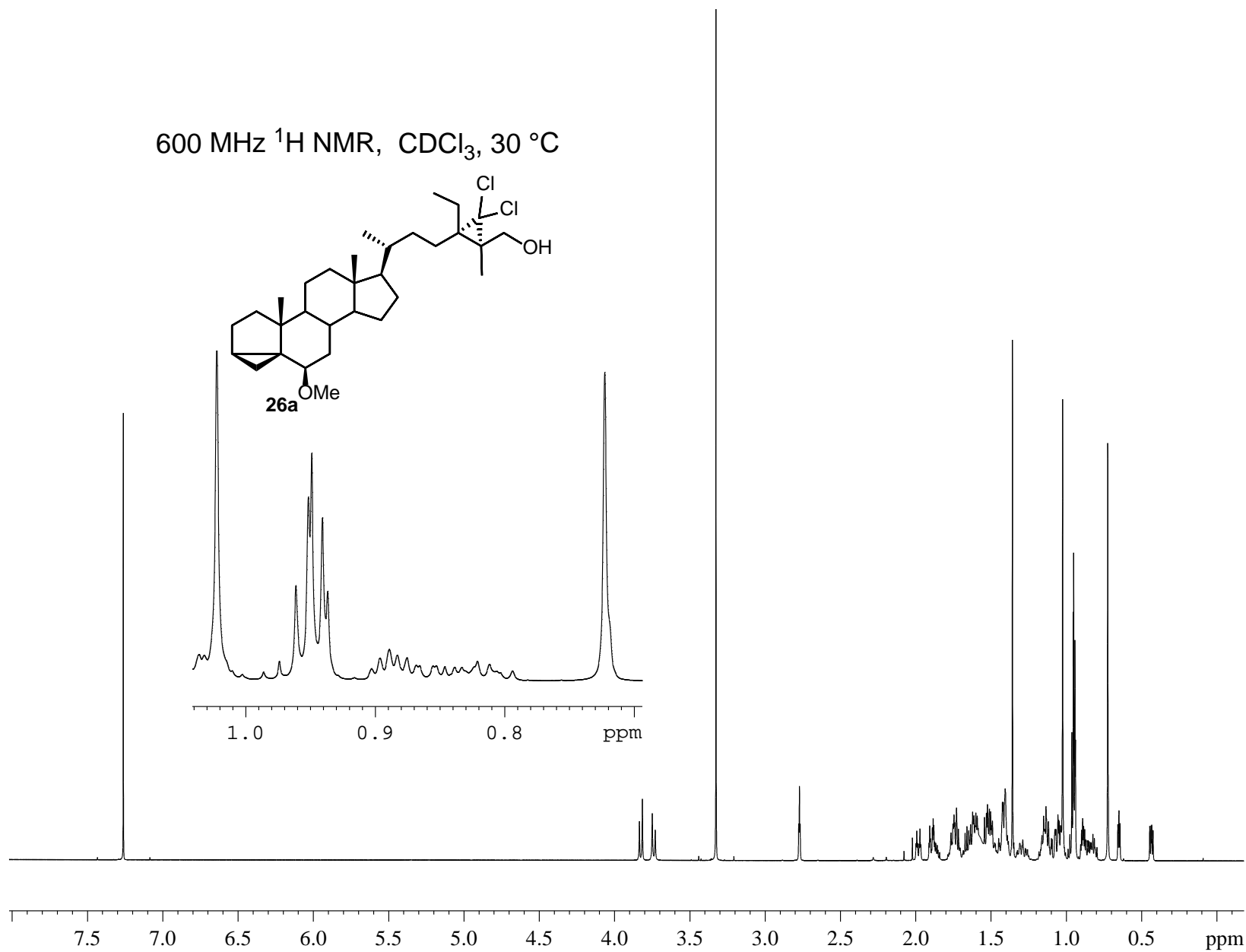
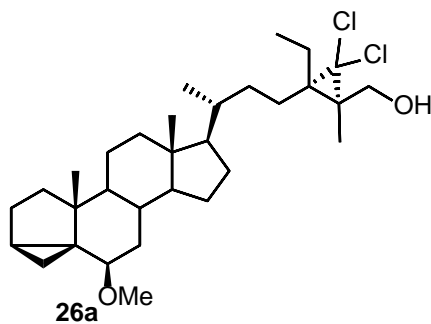
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

25b

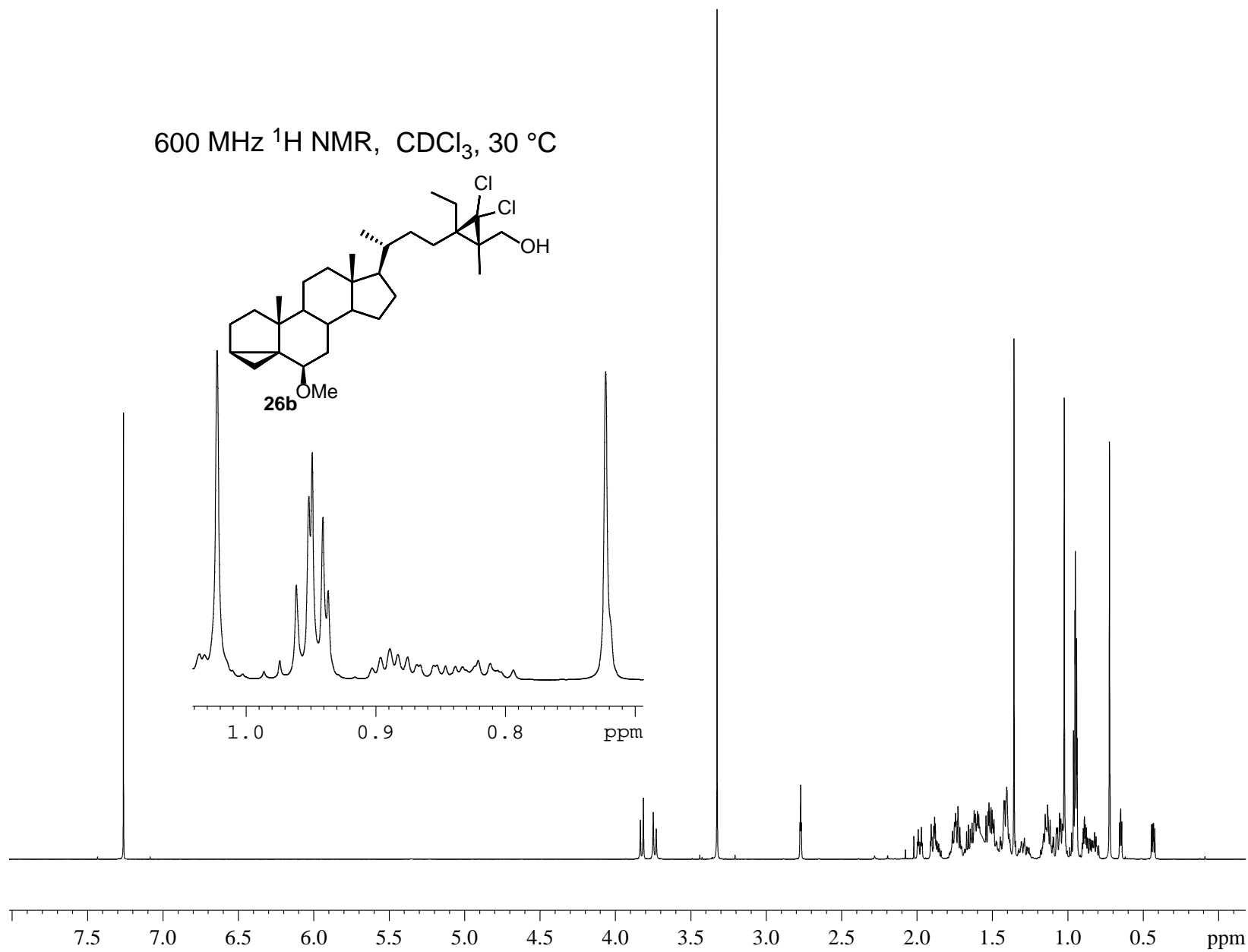
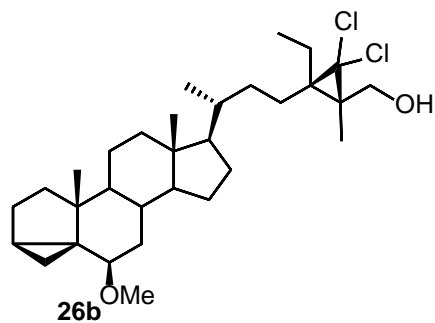
Chemical structure of **25b** is shown above the inset. The structure is a steroid derivative with a methoxy group (OMe) at C-3, a side chain containing a hydroxyl group (OH) and two chlorine atoms (Cl), and a methyl group (Me) at C-10.

The main spectrum shows peaks in the aromatic region (6.5-7.5 ppm), a cluster of peaks between 0.5 and 2.5 ppm, and a sharp peak at approximately 3.9 ppm. The inset shows a detailed view of the 0.8-1.1 ppm region, with peaks labeled at 1.0, 0.9, and 0.8 ppm.

600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



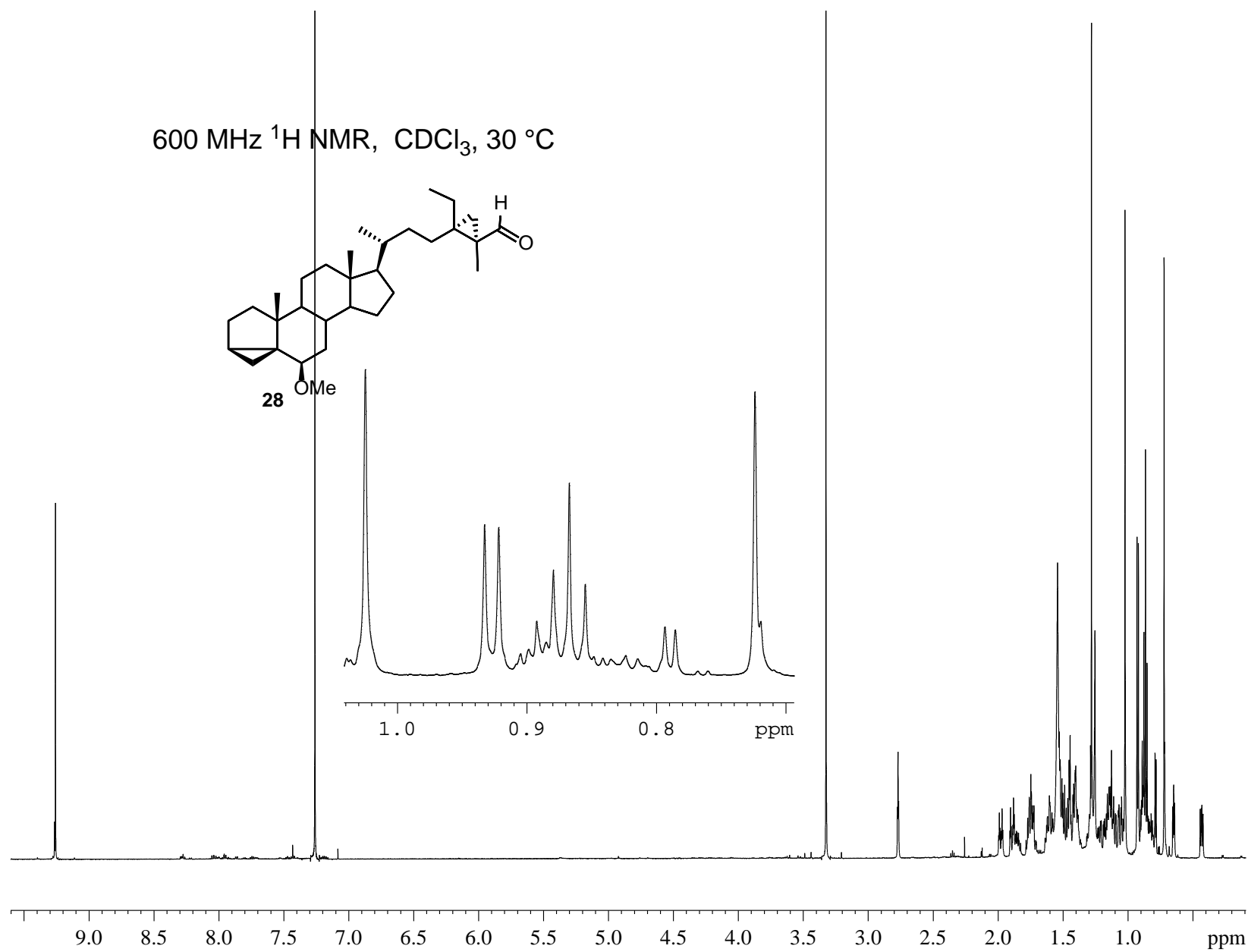
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



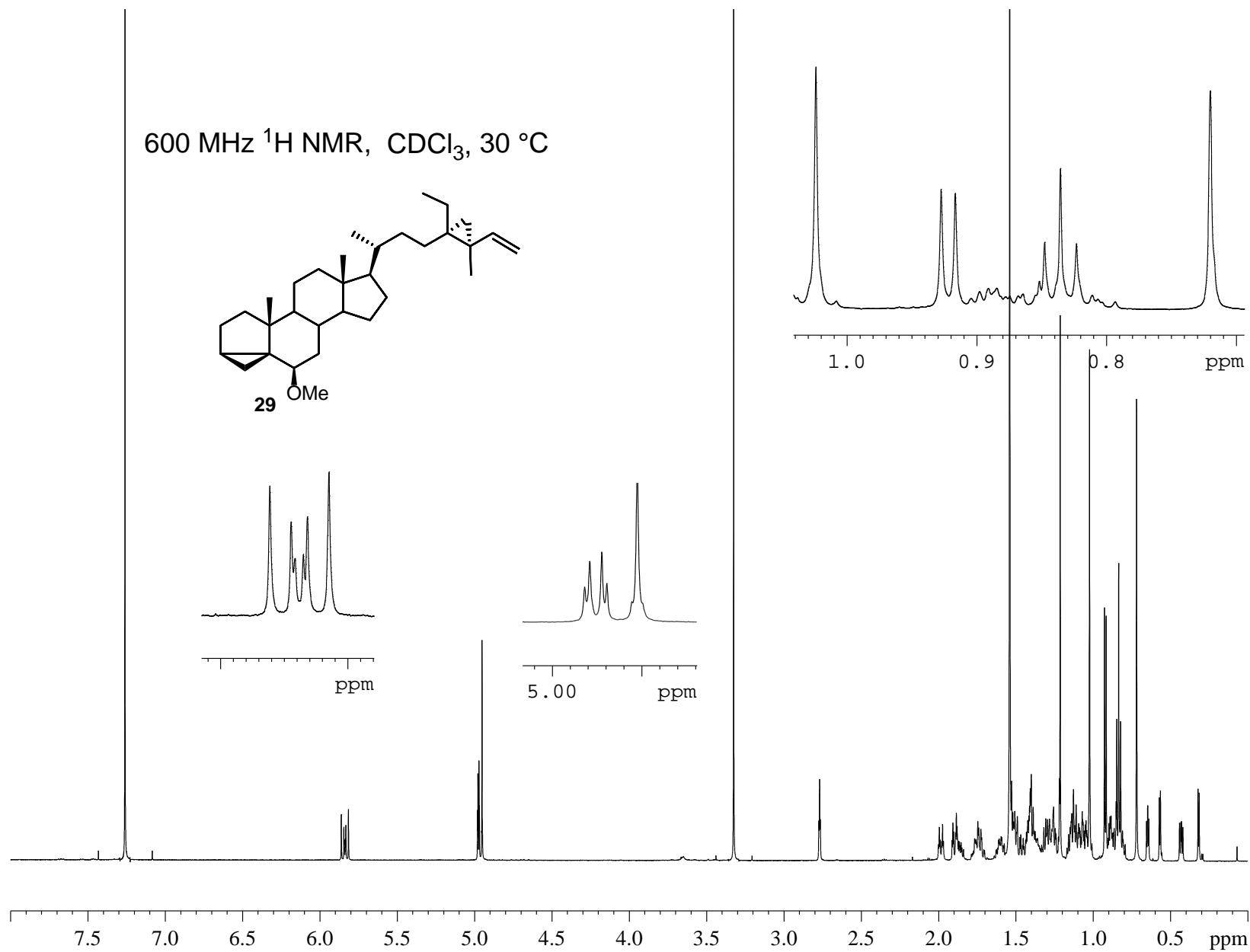
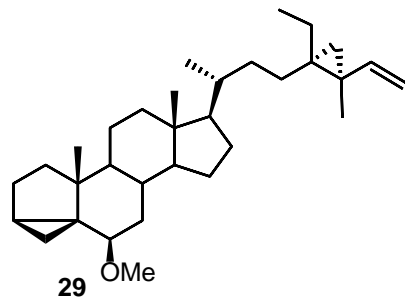
800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

Chemical structure of compound 27 is shown above the spectrum. The structure is a complex polycyclic molecule with a steroid-like core, featuring a methoxy group (OMe) and a side chain with a hydroxyl group (OH).

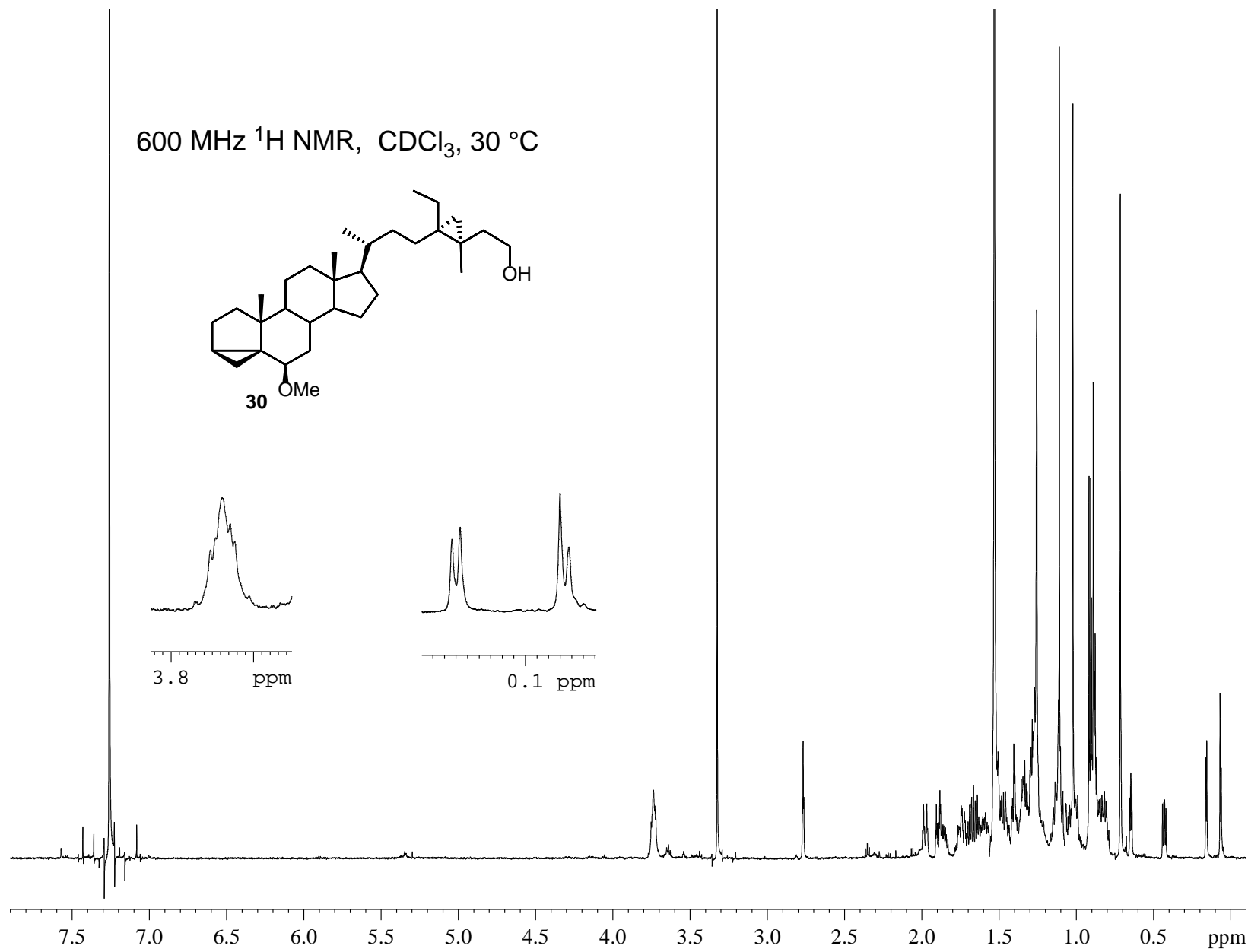
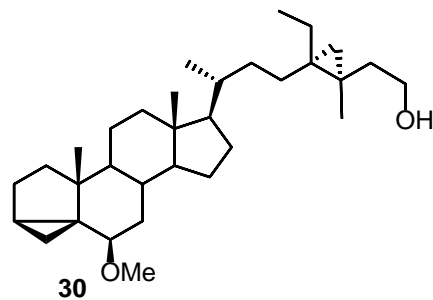
The spectrum displays peaks in the aromatic region (6.5-7.5 ppm), a complex set of peaks in the aliphatic region (0.5-6.0 ppm), and a sharp peak at approximately 3.8 ppm. An inset zooms in on the 0.8-1.1 ppm region, showing several sharp peaks.

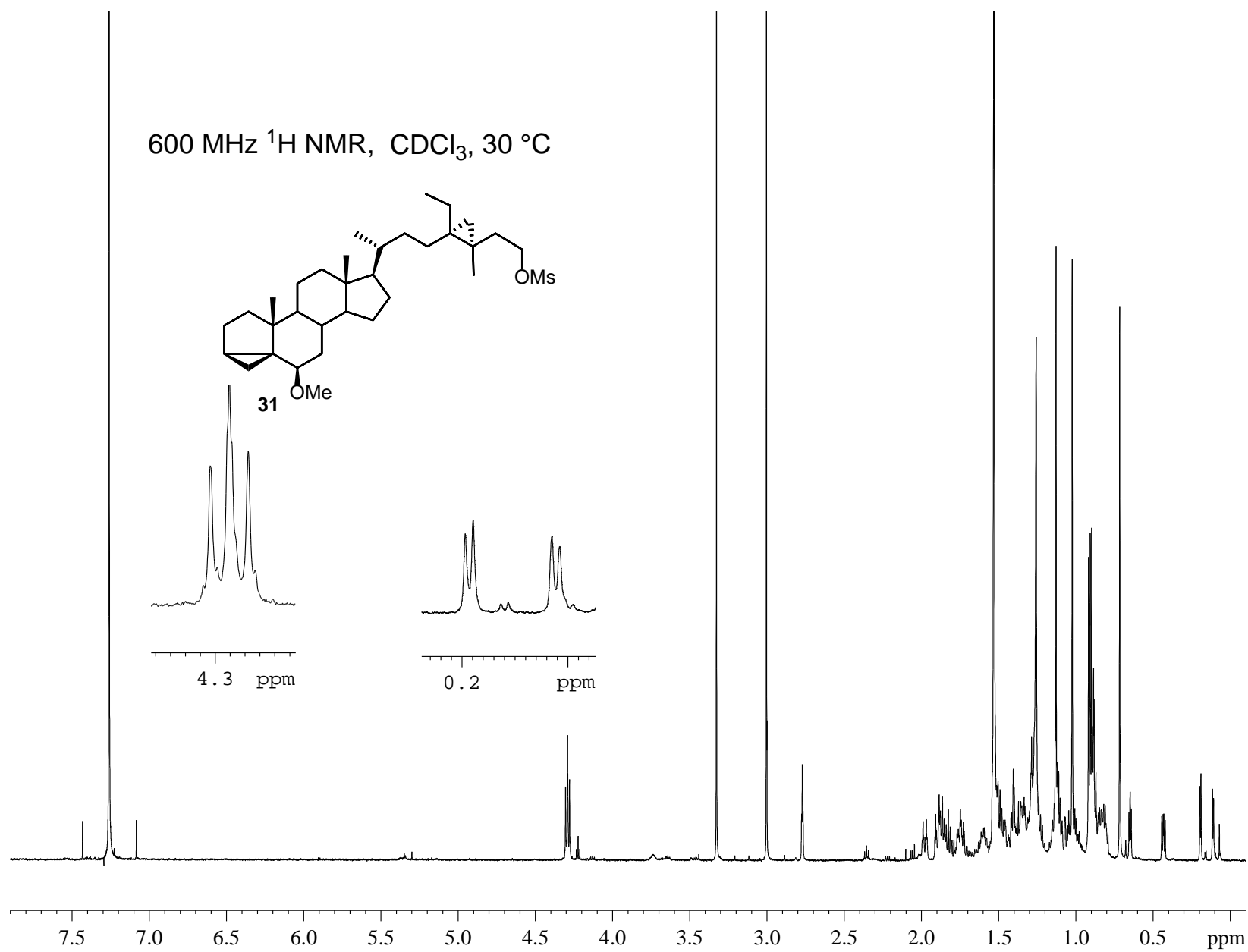


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

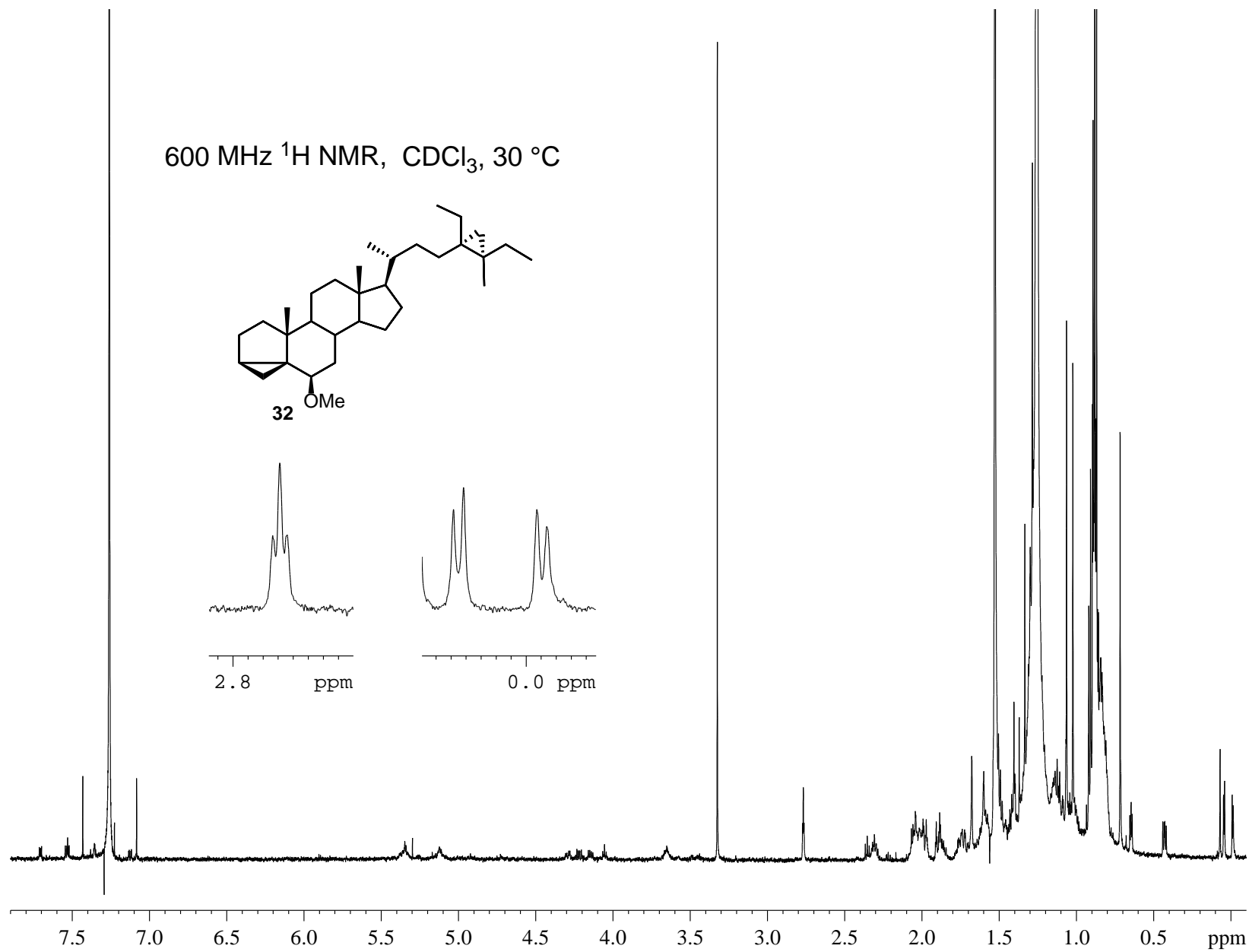
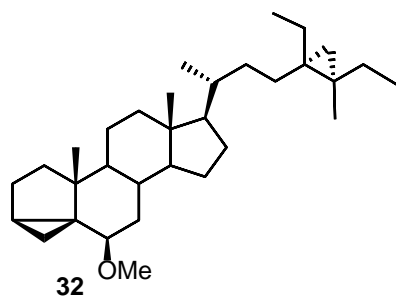


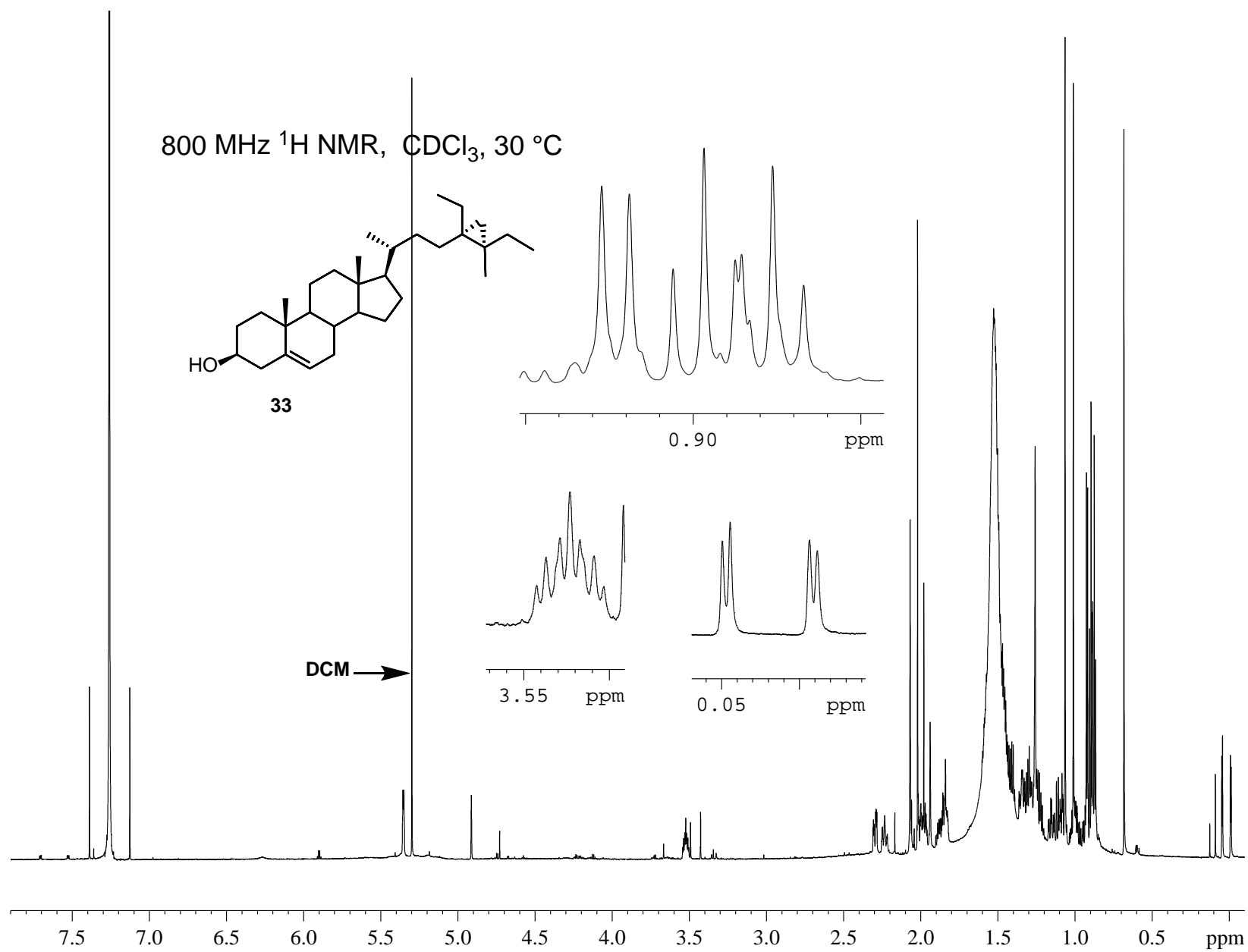
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



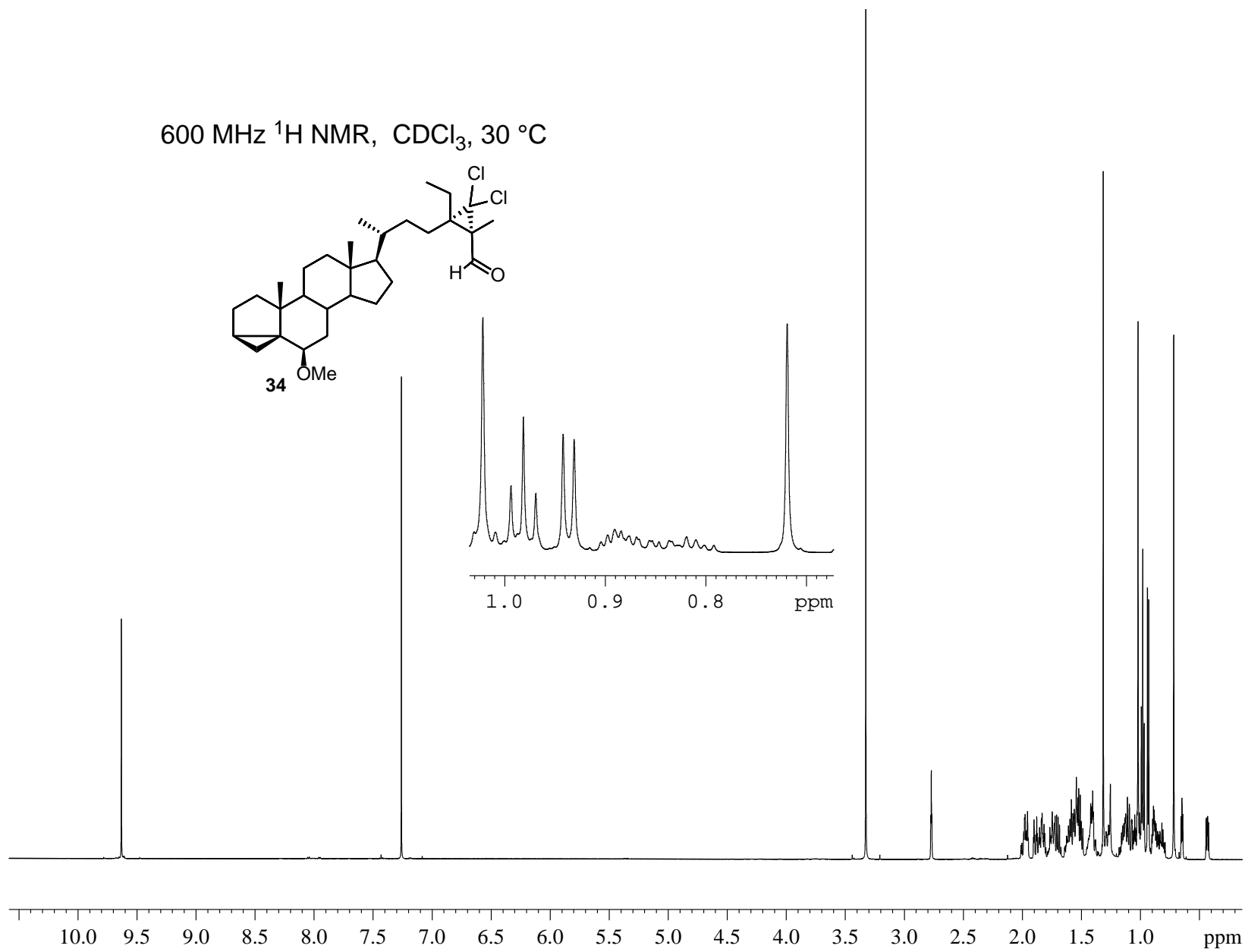
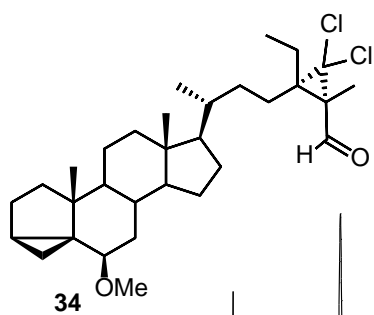


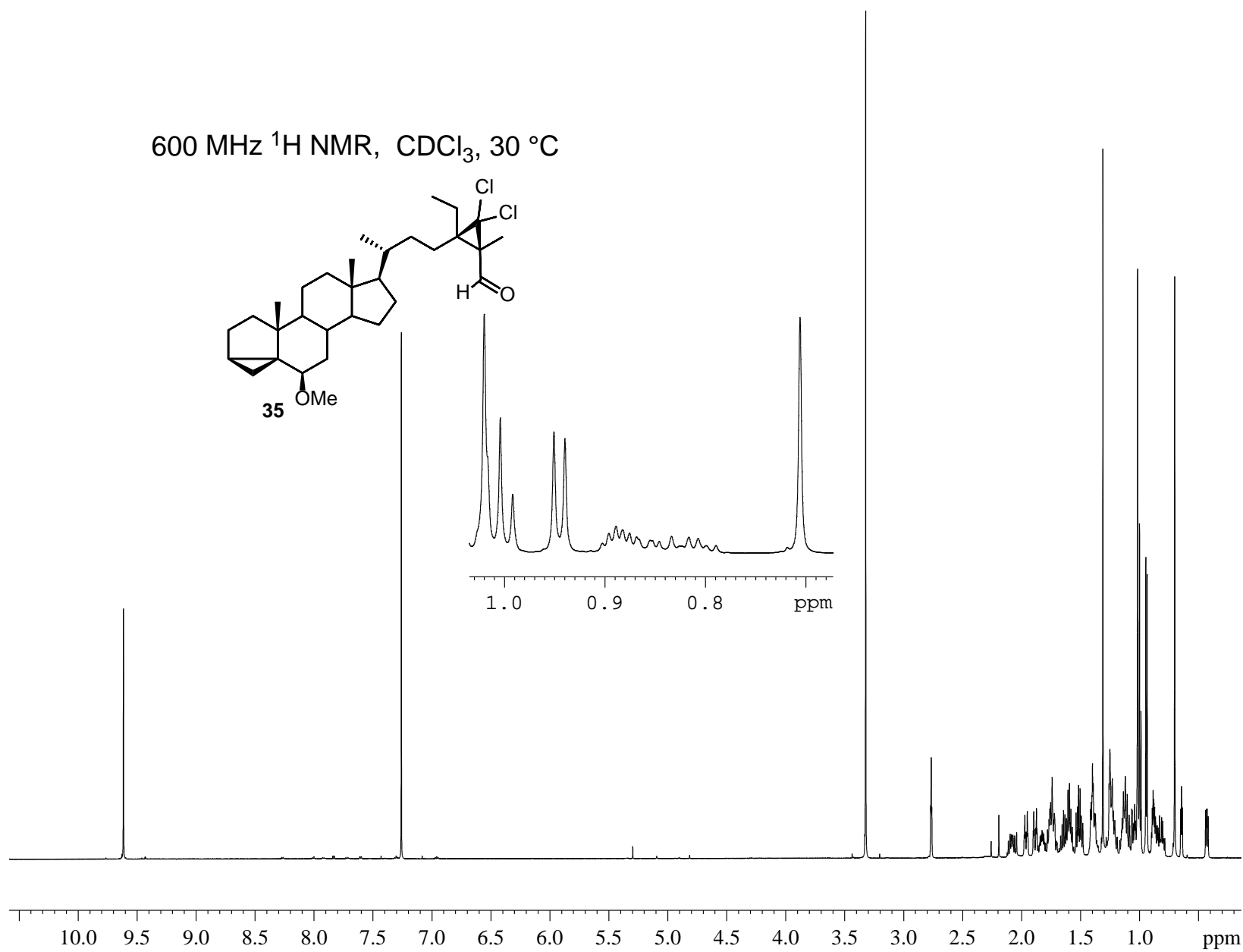
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

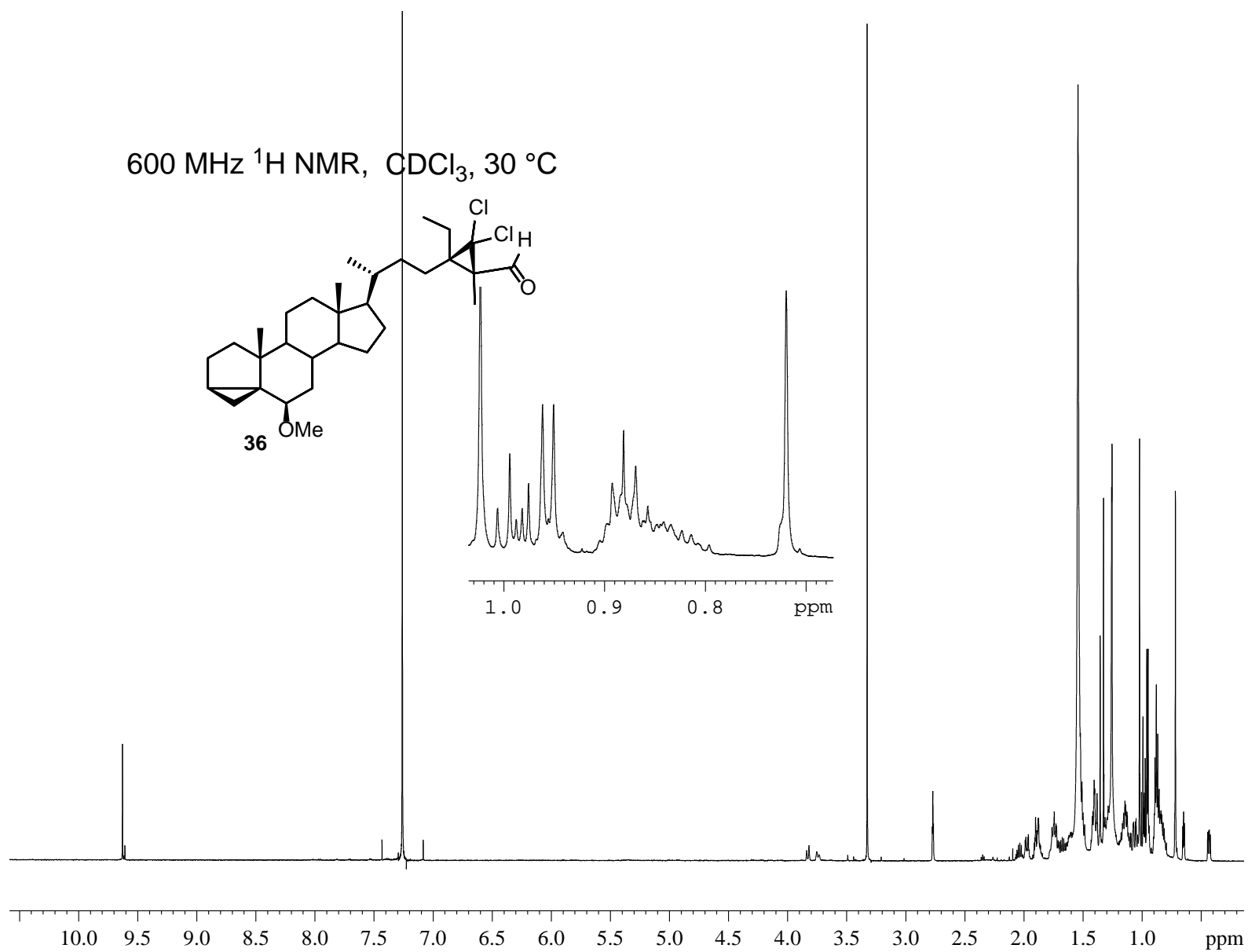


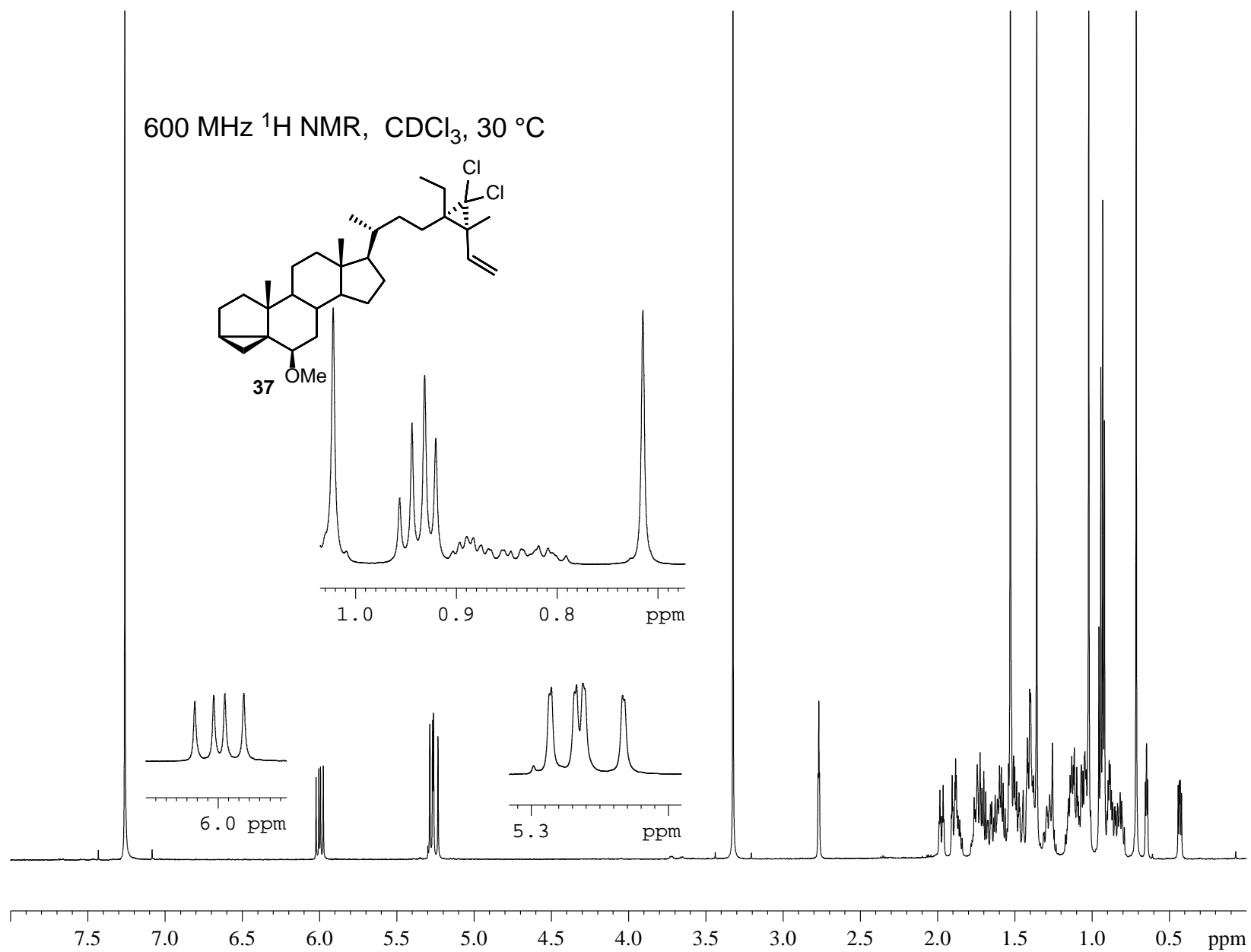


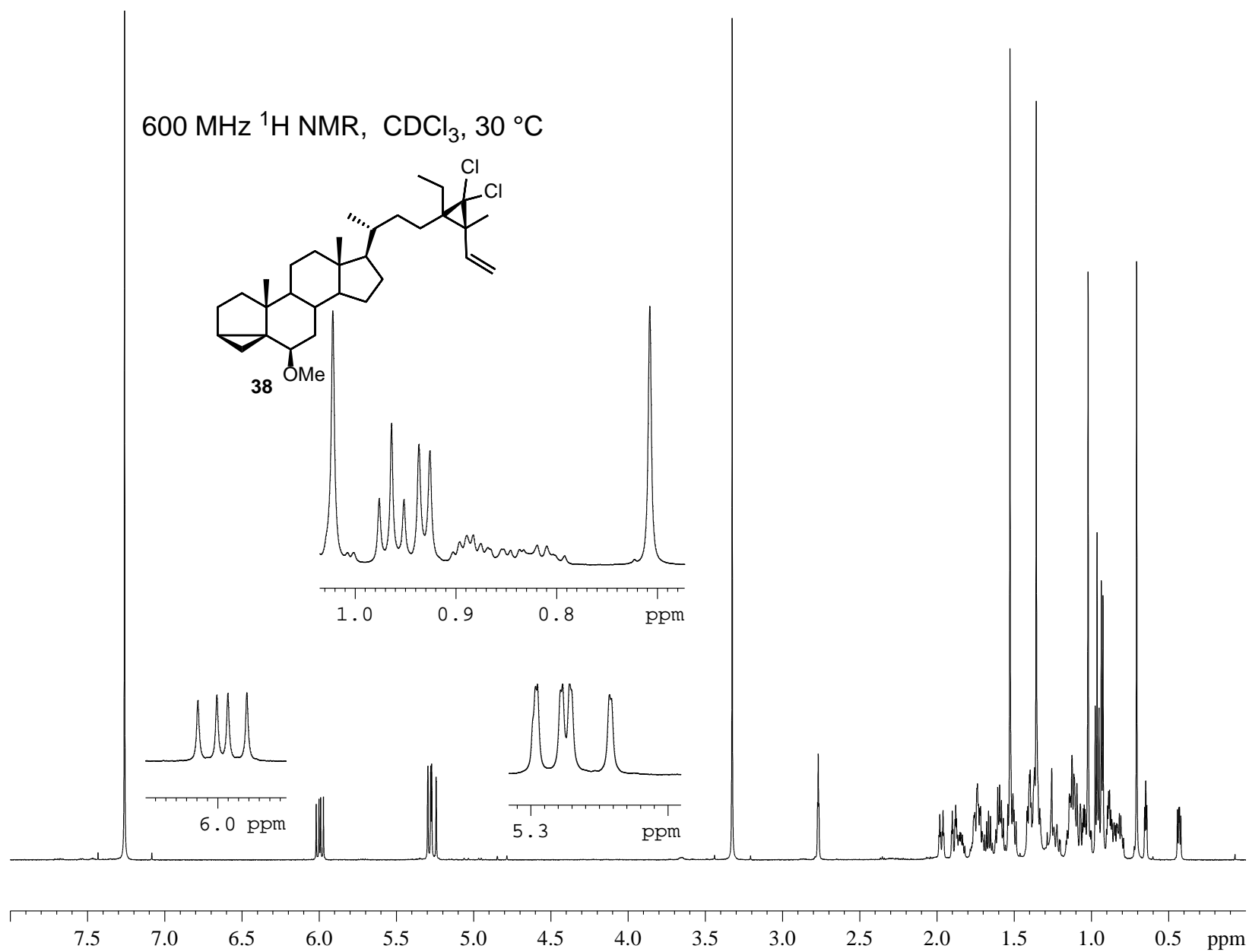
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

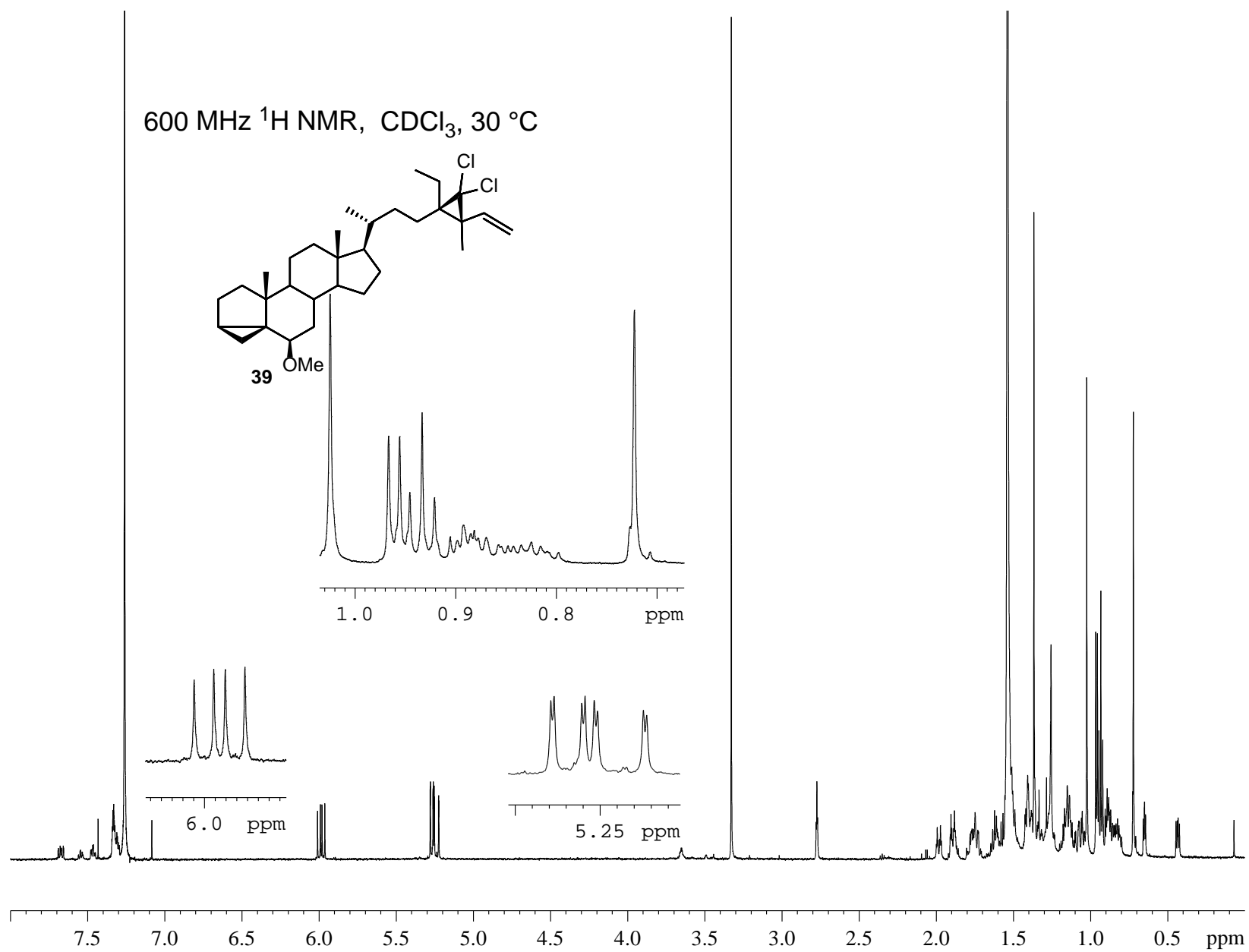


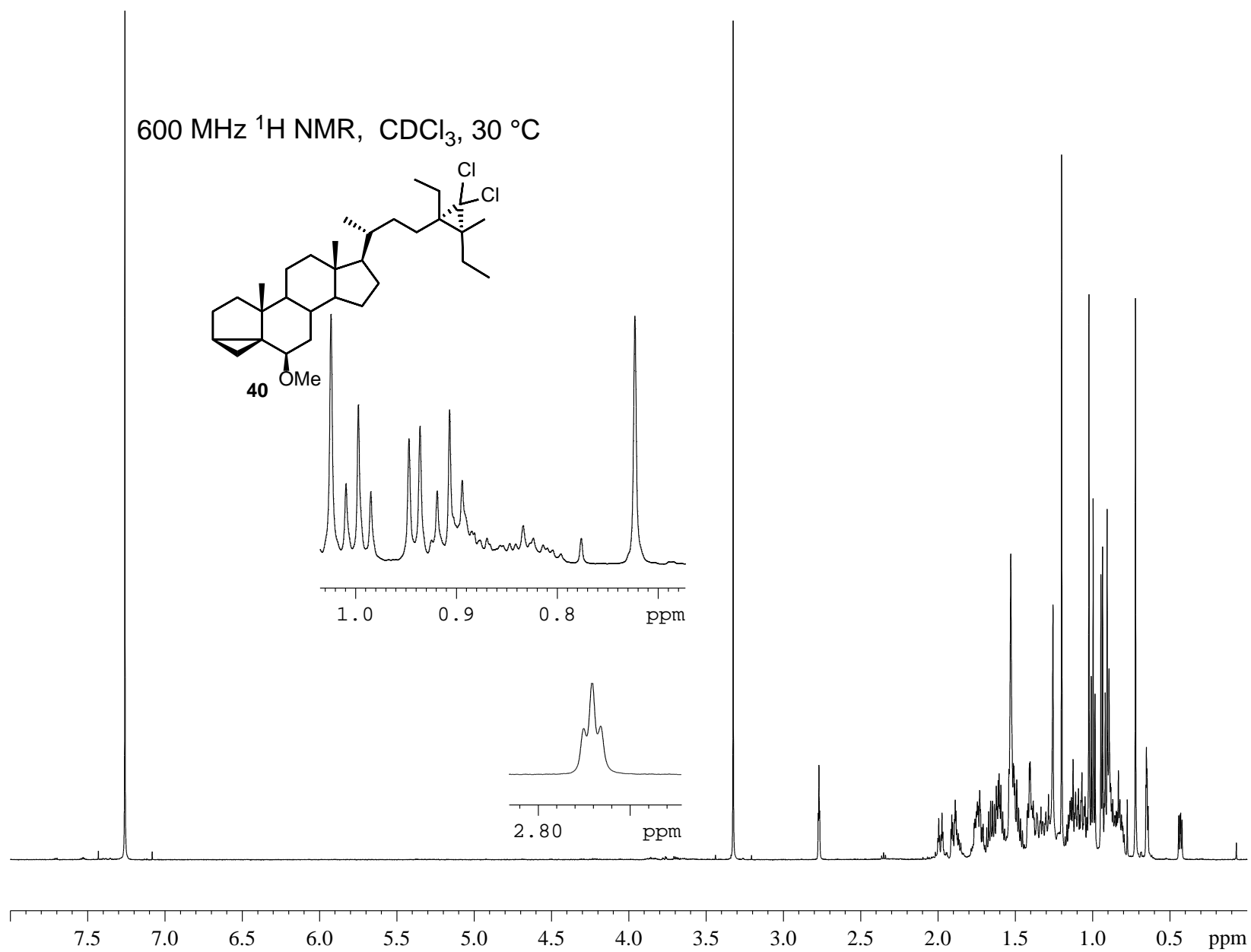


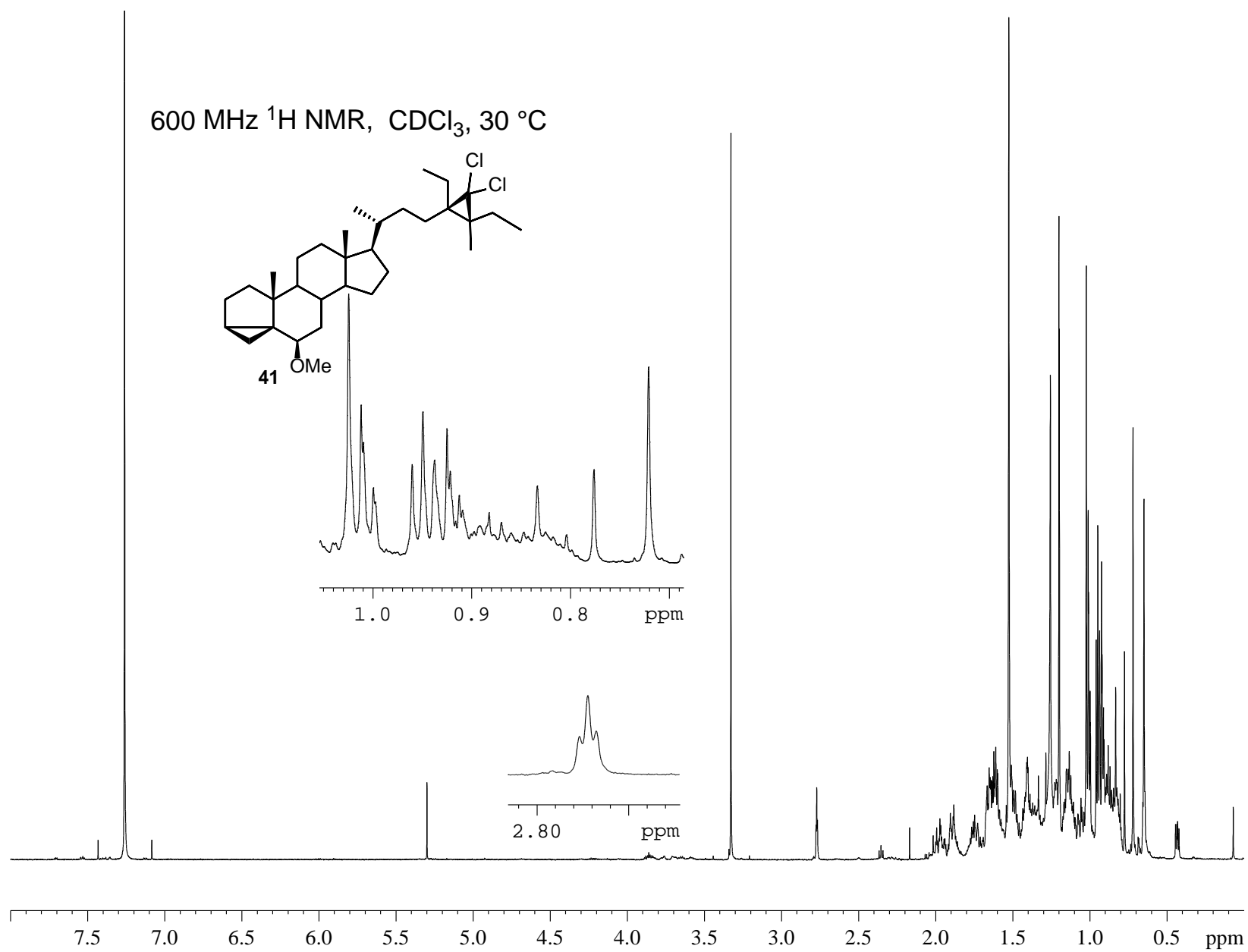


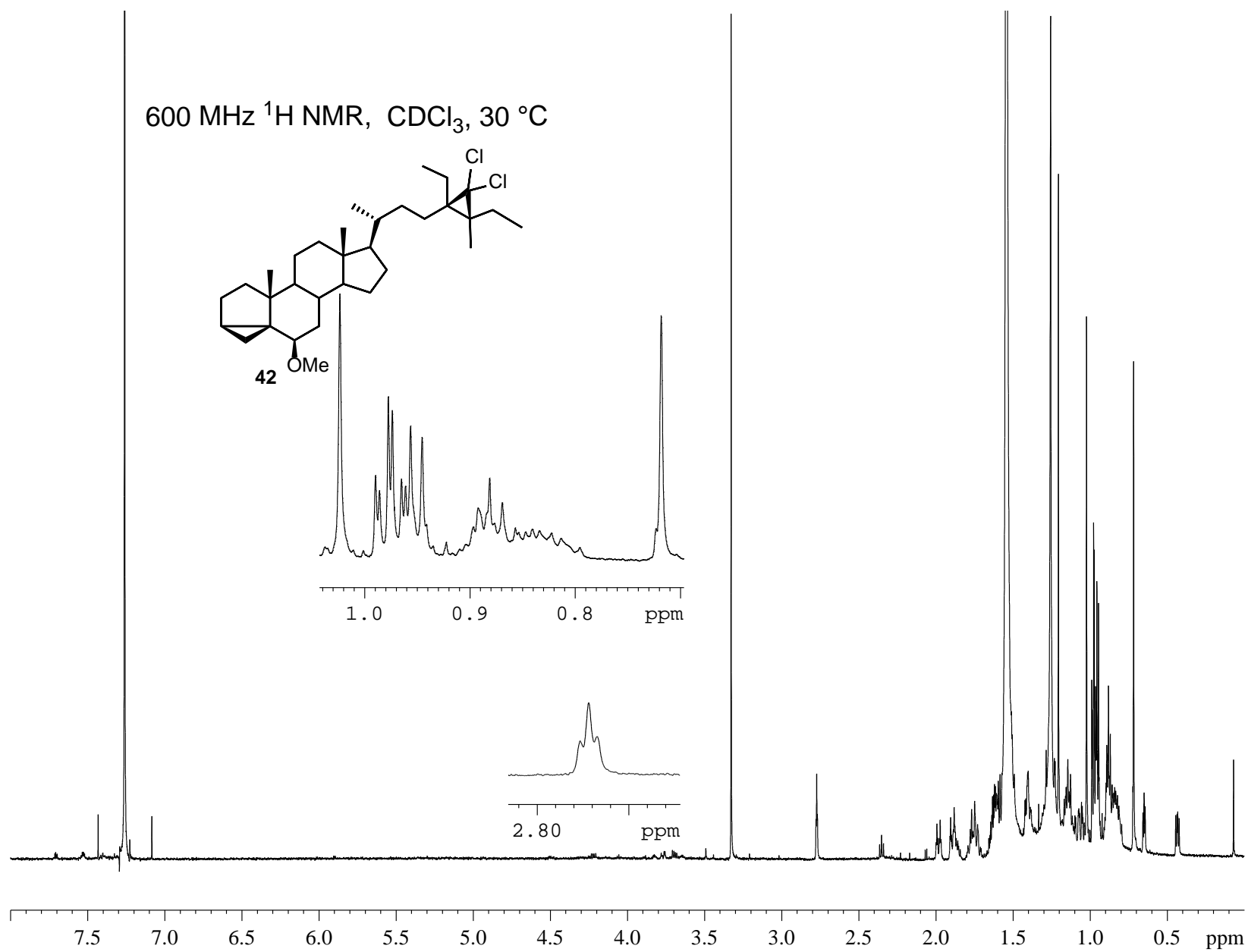


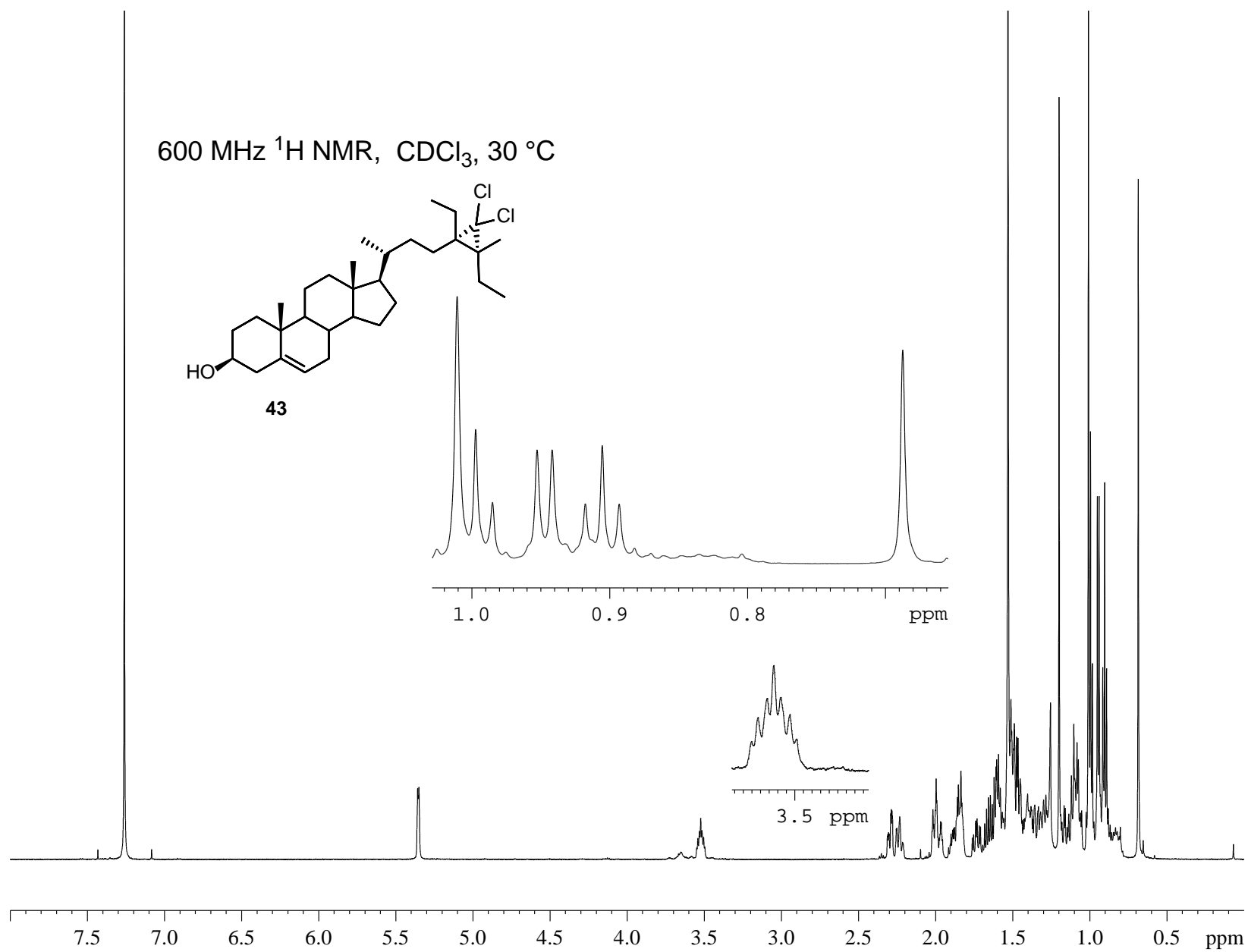


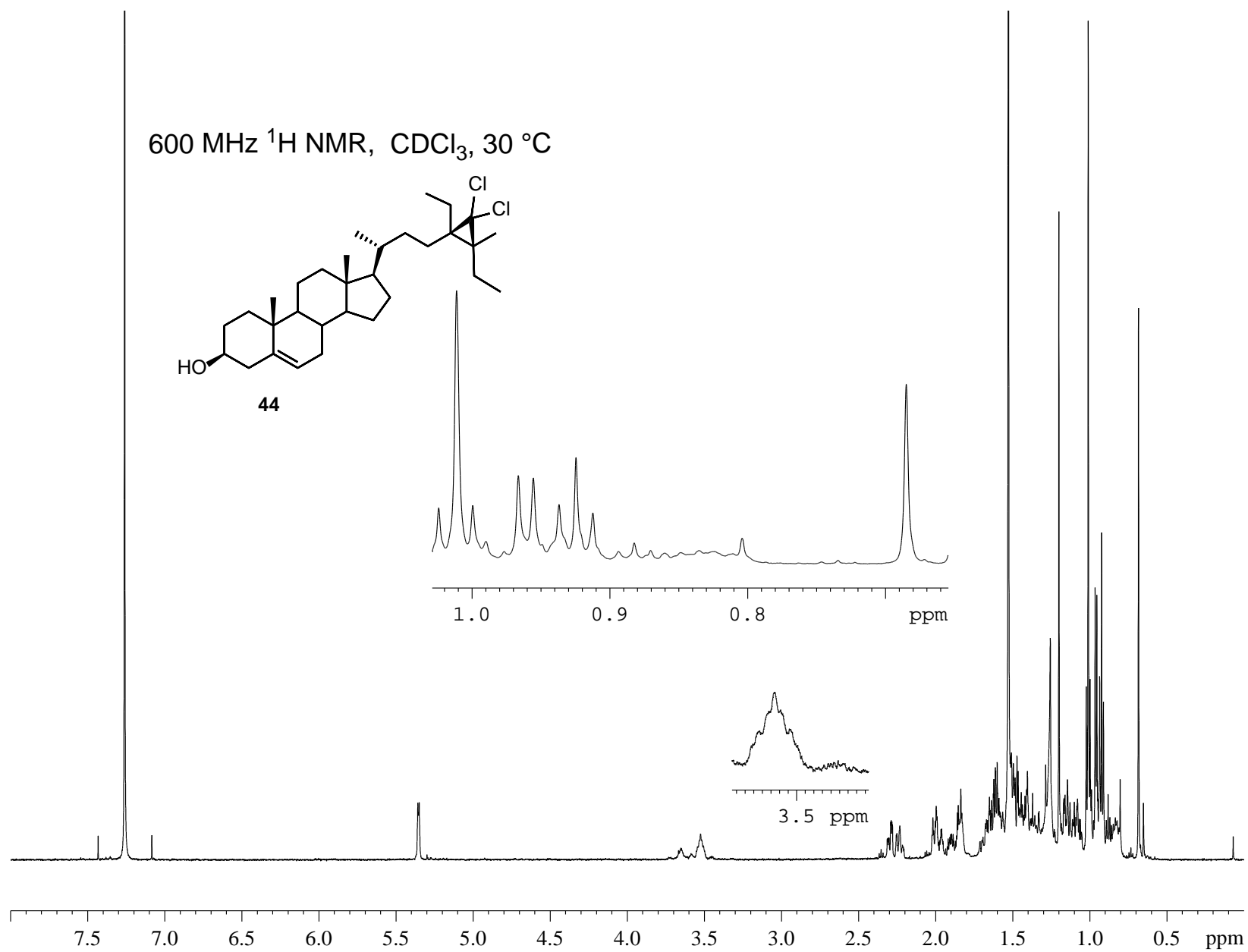


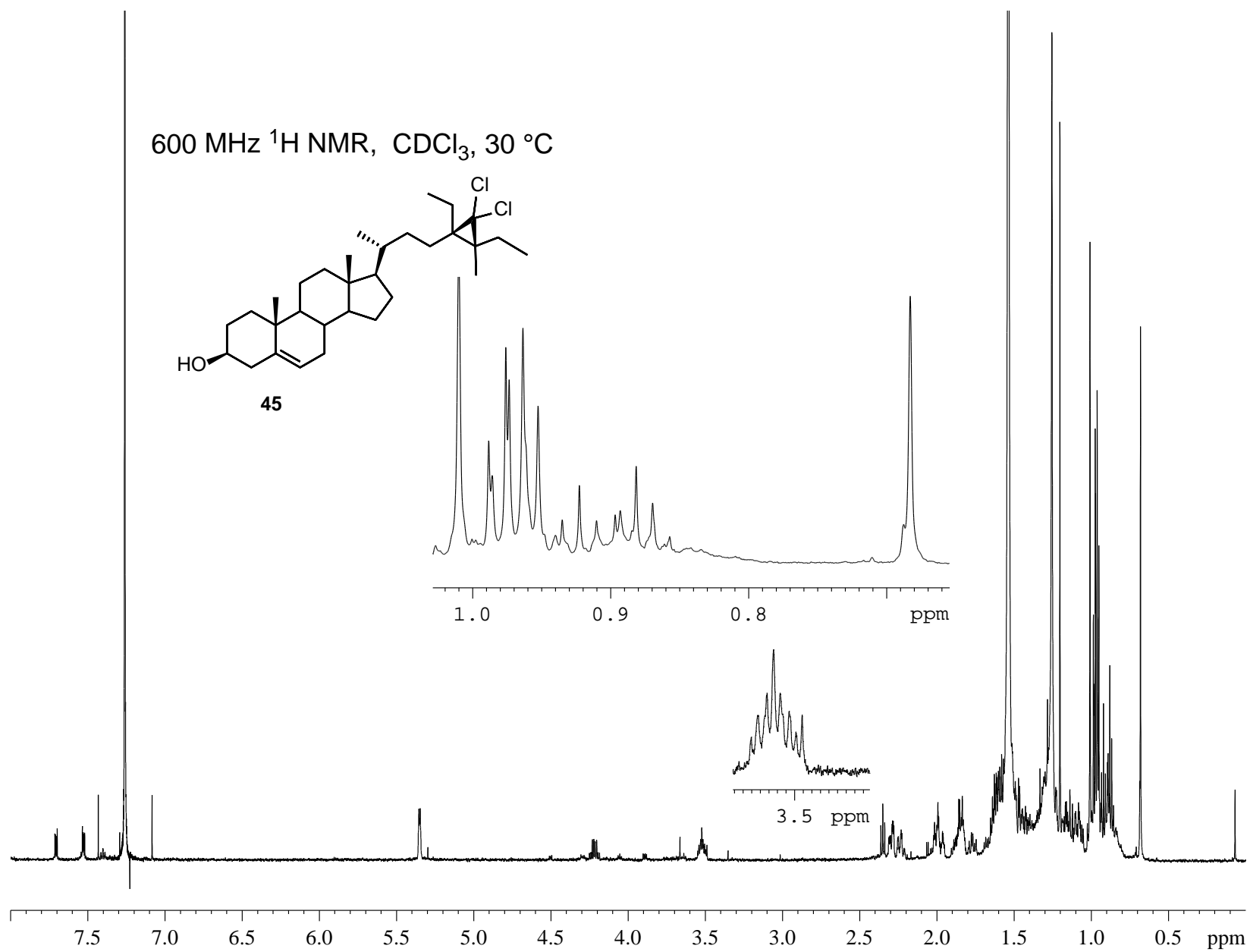




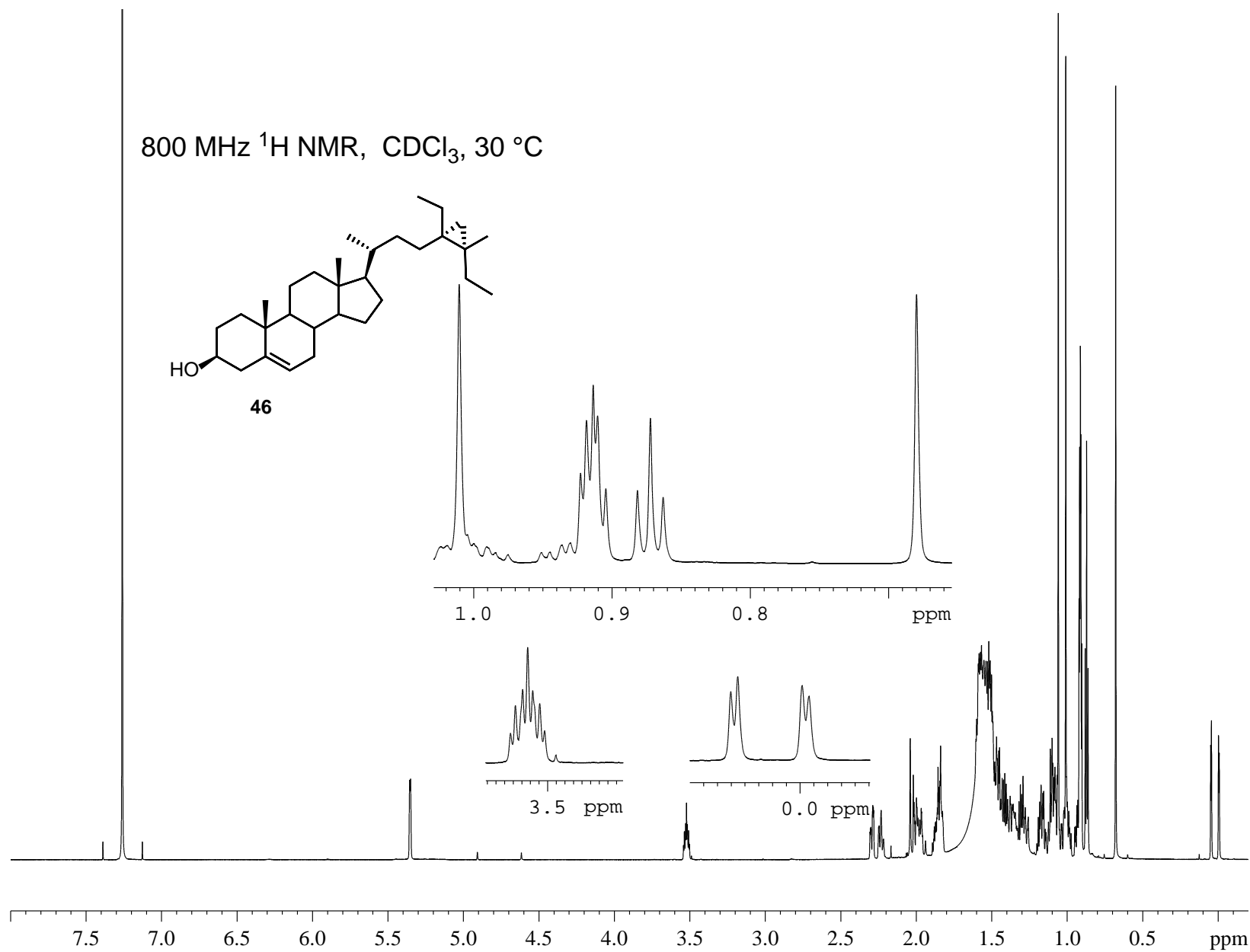
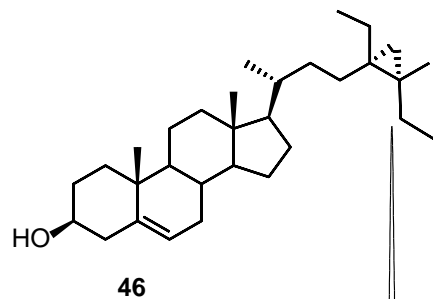




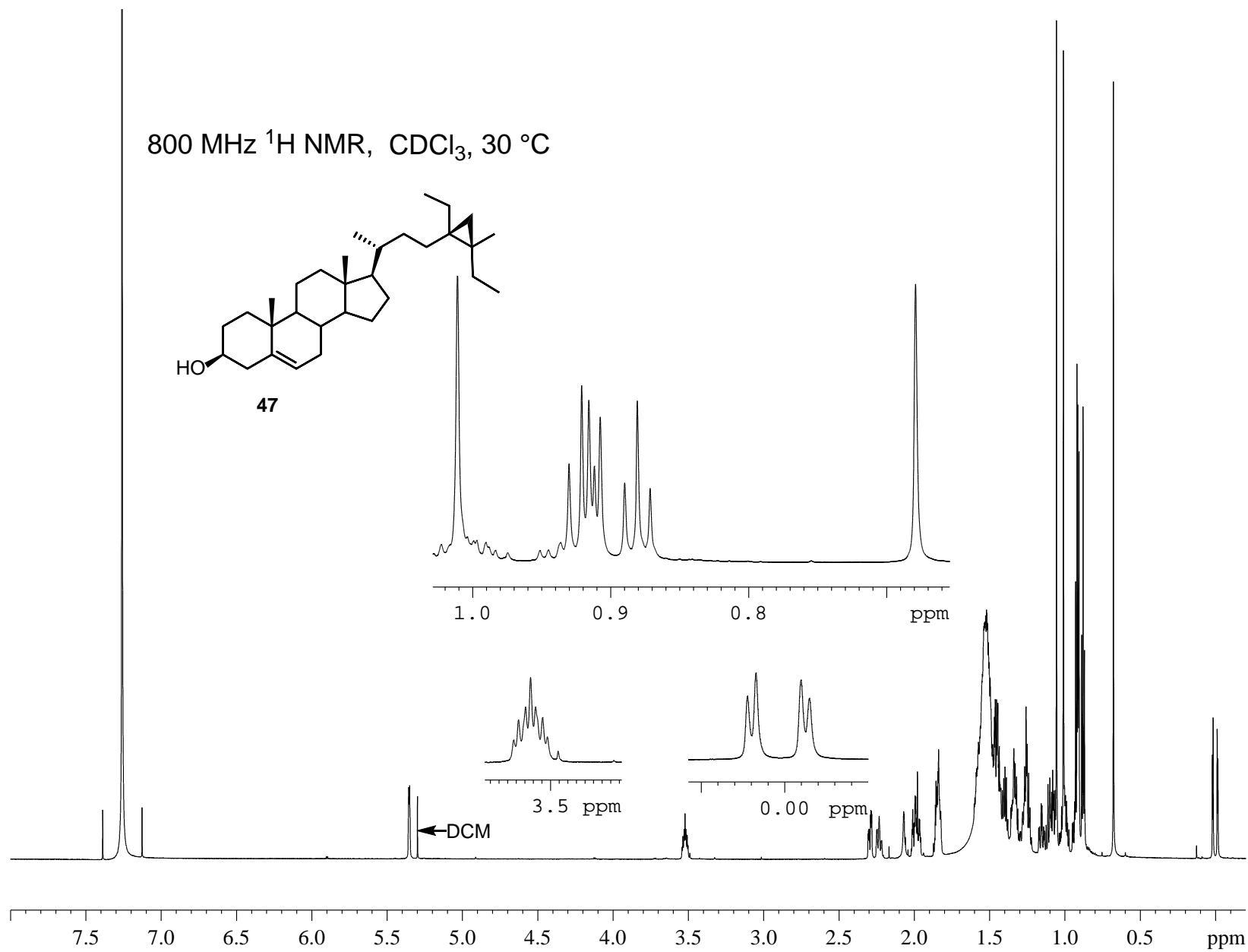
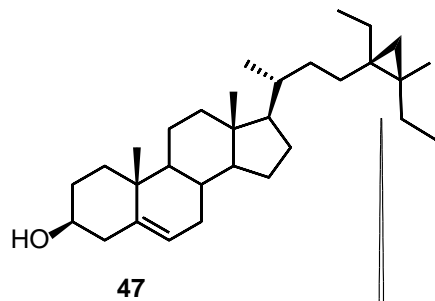




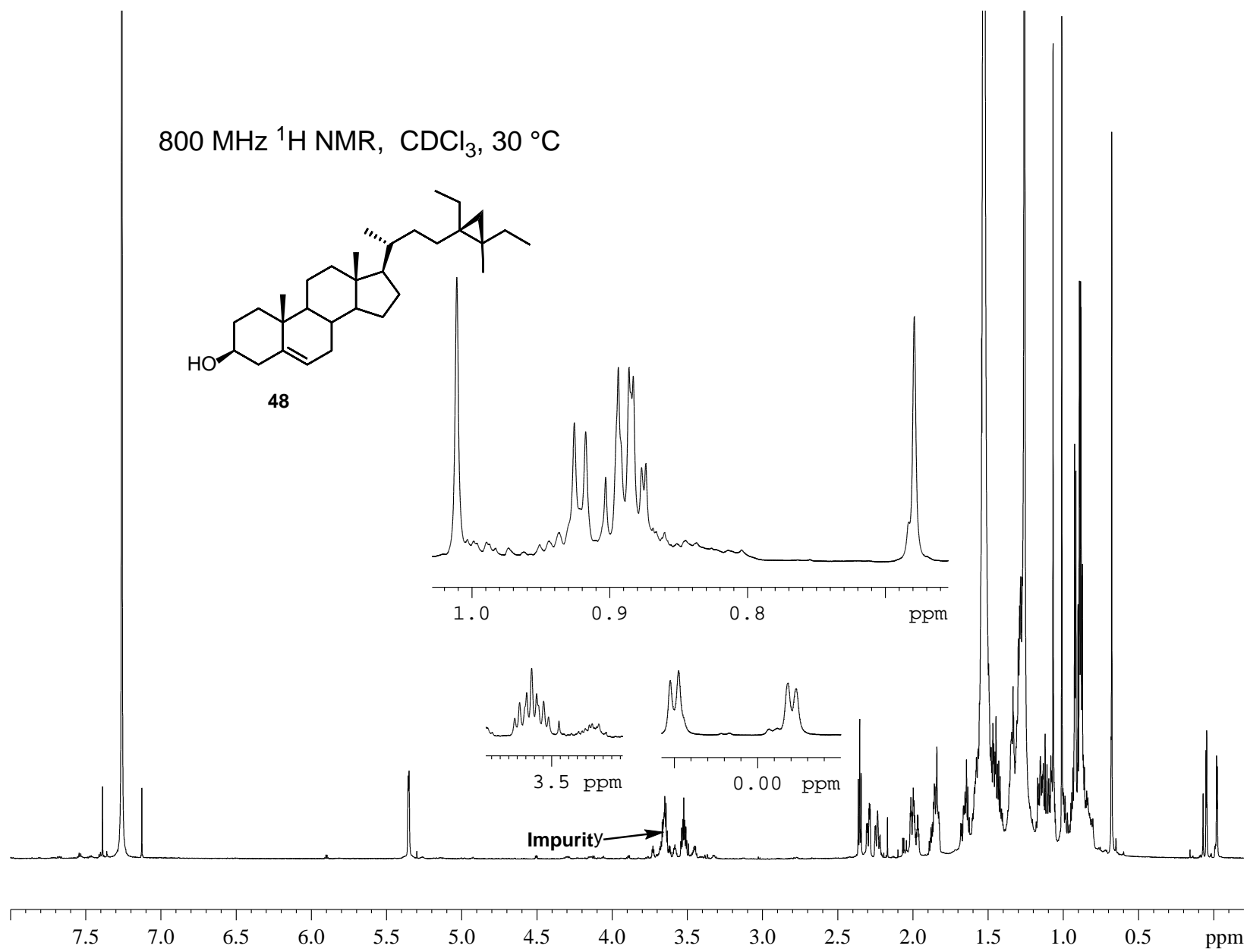
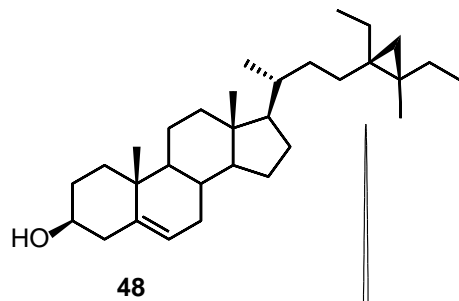
800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

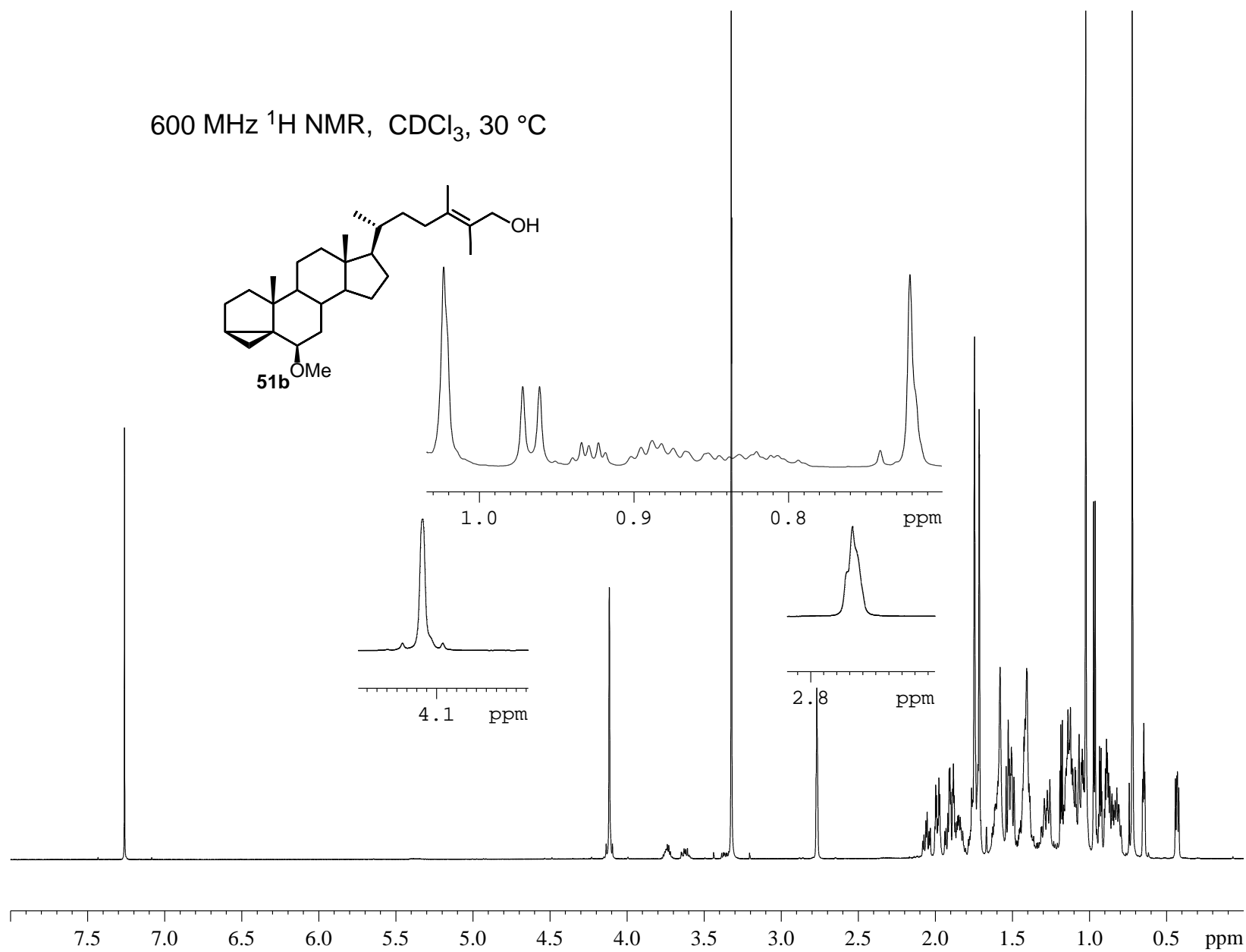
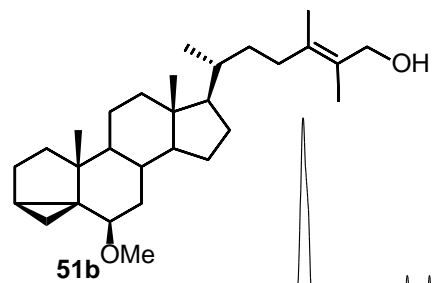
51a

1.0 0.9 0.8 ppm

4.1 ppm

2.8 ppm

600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

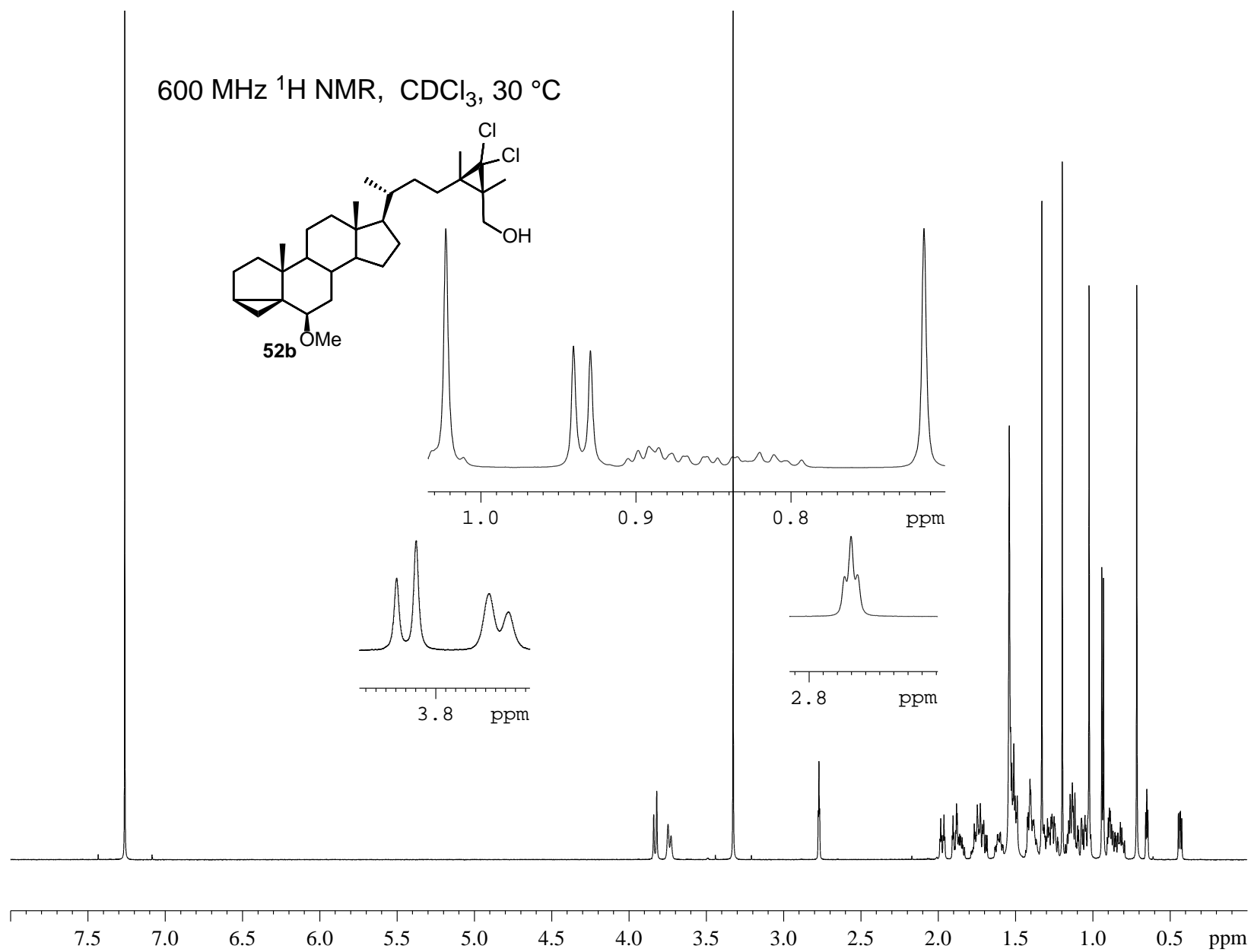


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

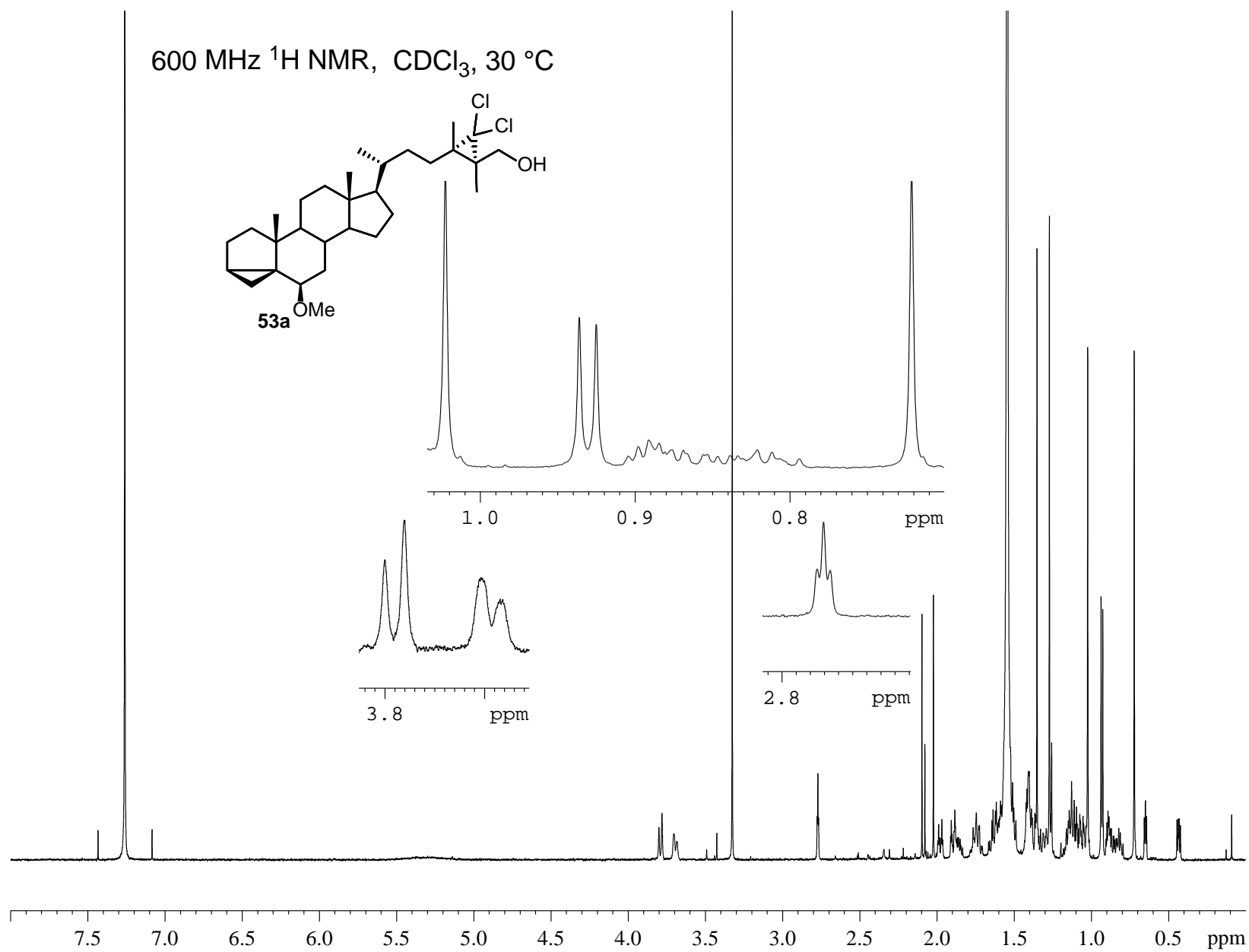
52a

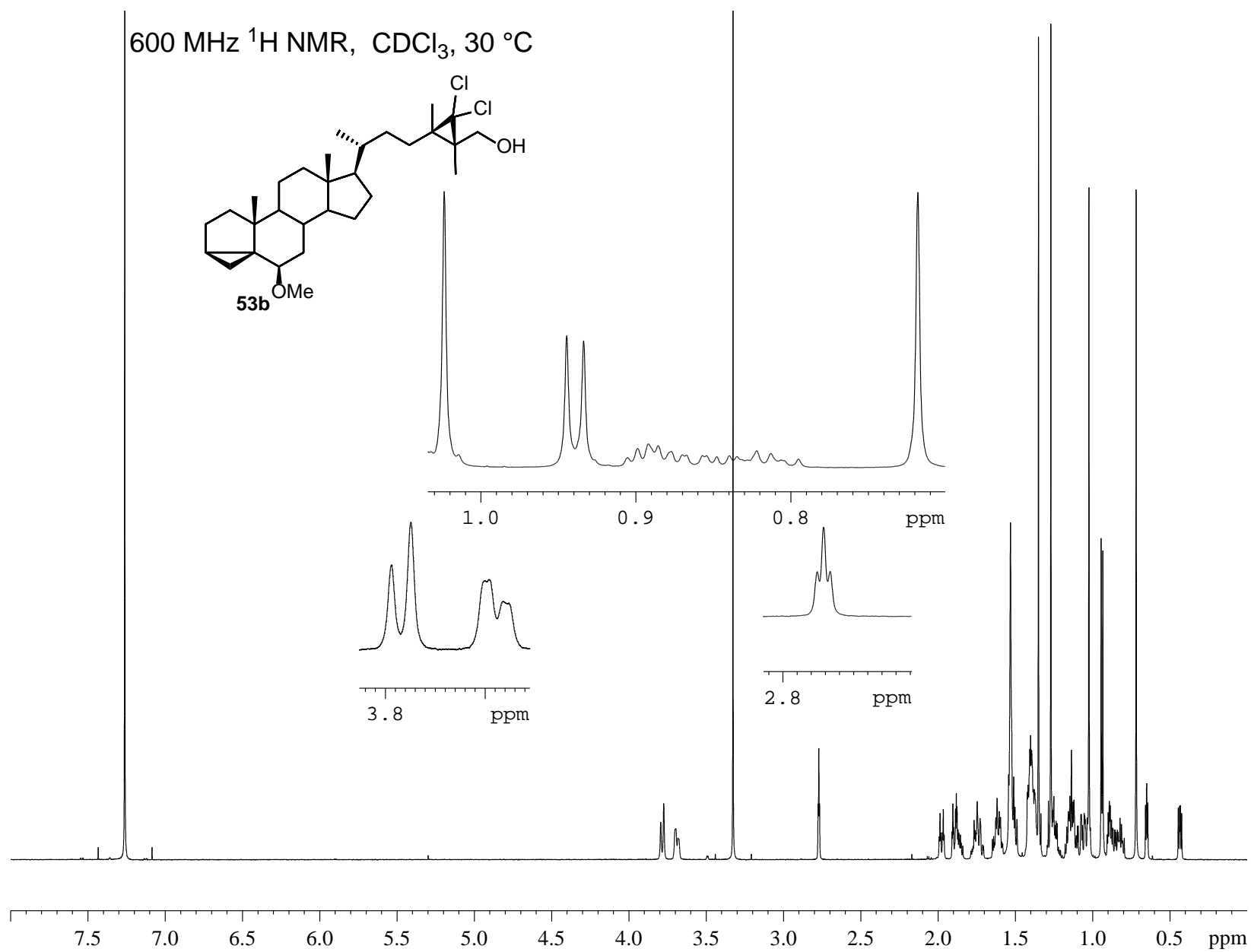
Chemical structure of **52a** is shown, featuring a steroid-like core with a methoxy group (OMe) and a side chain containing a 1,1-dichloroethyl group and a hydroxyl group (OH).

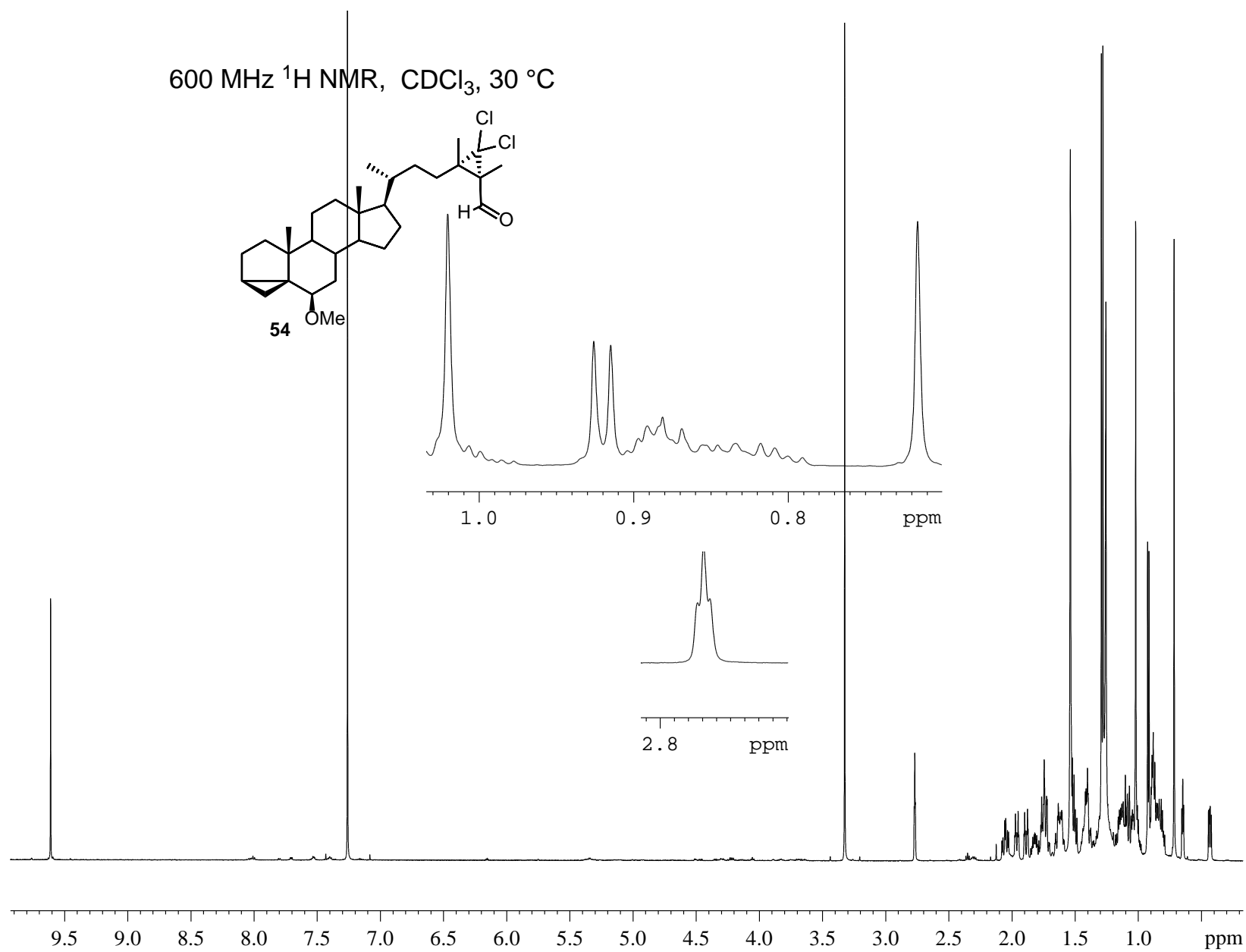
The NMR spectrum displays peaks corresponding to the structure, with chemical shifts ranging from approximately 0.5 ppm to 7.5 ppm. Key peaks include a methoxy singlet around 3.8 ppm, a diastereotopic methylene quartet around 2.7 ppm, a 1,1-dichloroethyl singlet around 2.1 ppm, and a complex aliphatic region between 0.5 and 1.5 ppm. Two insets provide expanded views of the 0.8-1.1 ppm and 3.7-3.9 ppm regions.



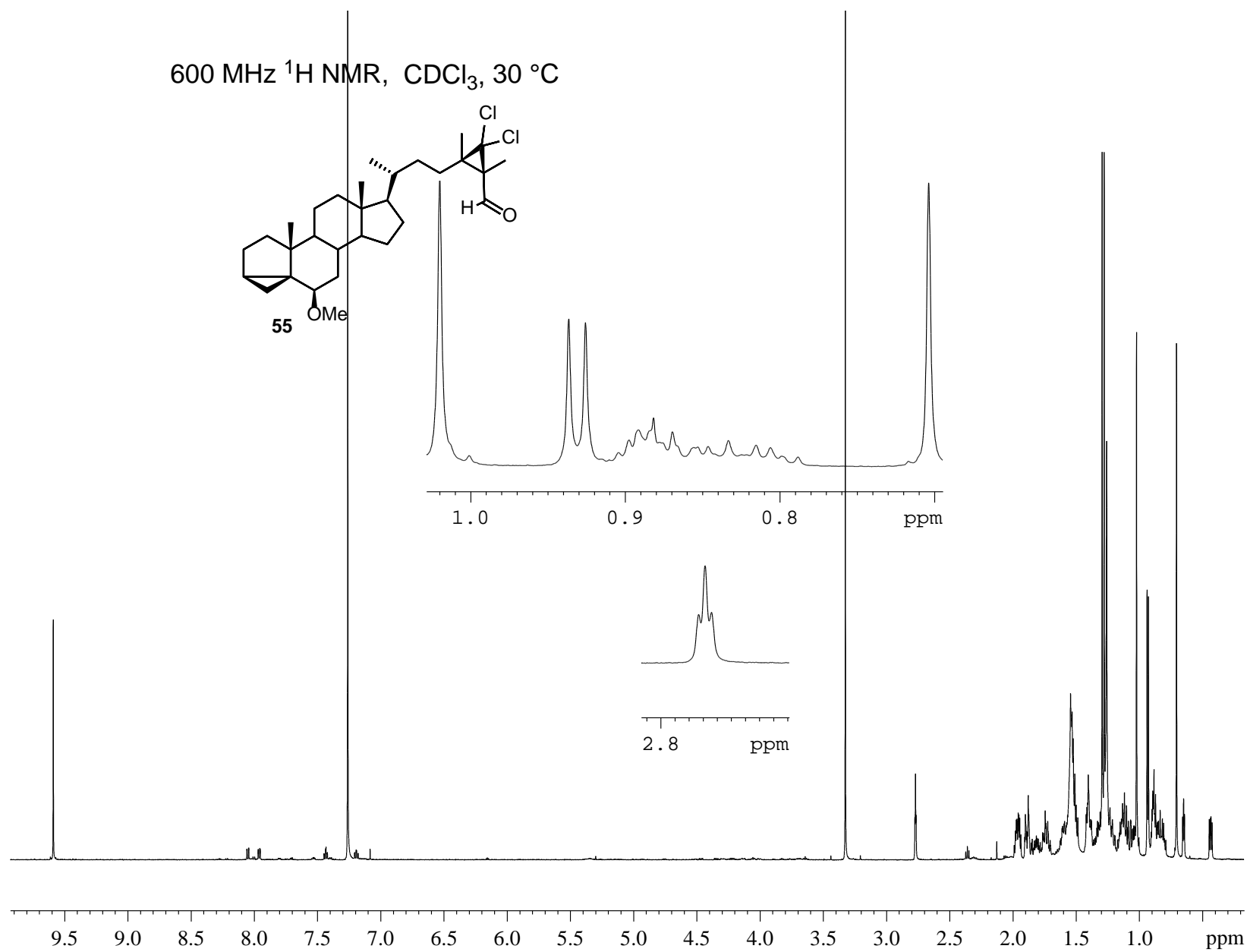
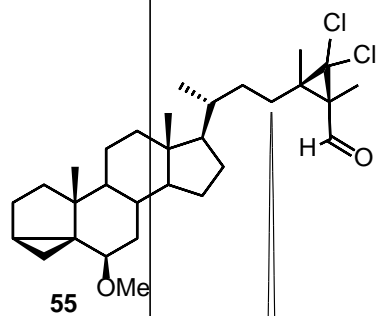
53a

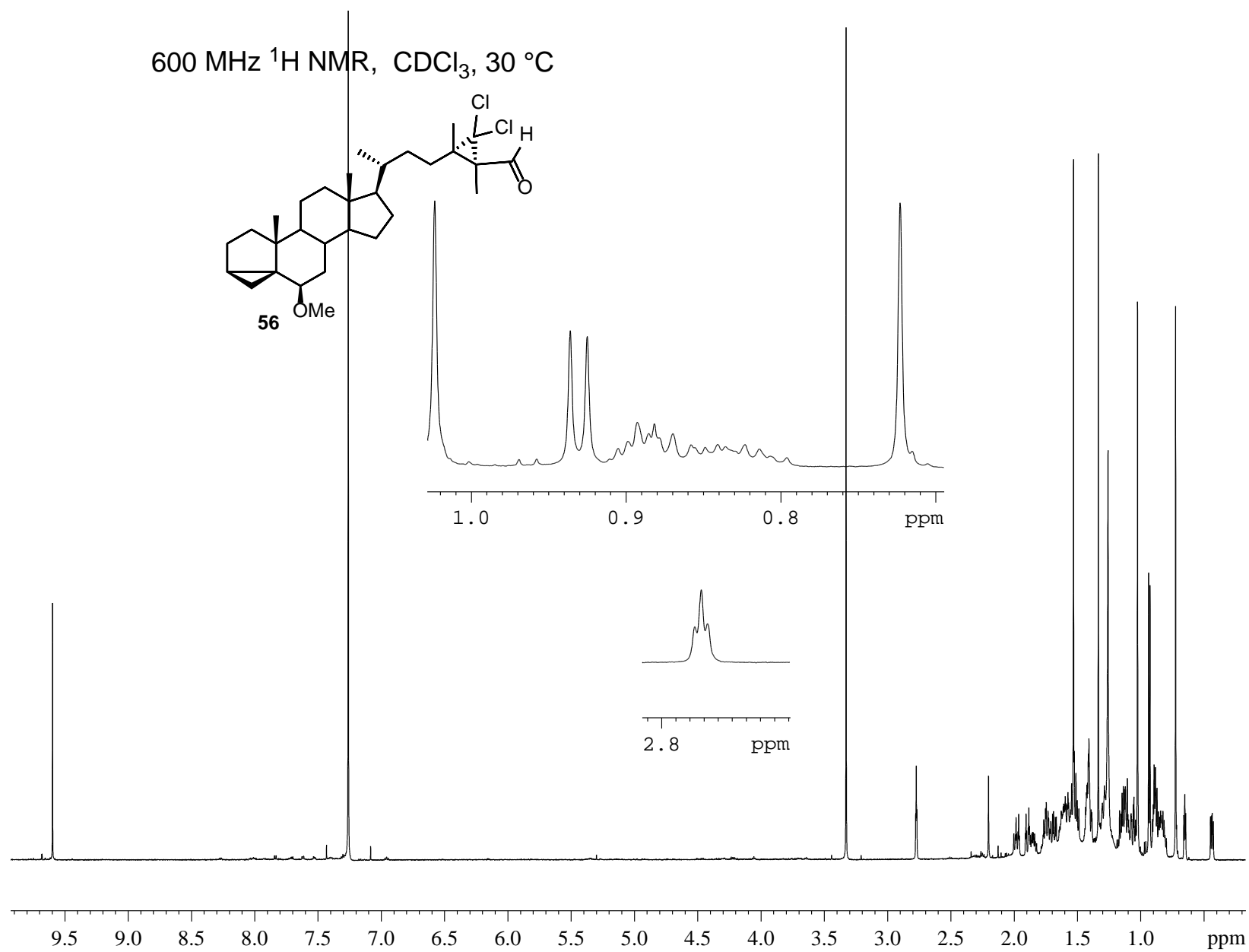


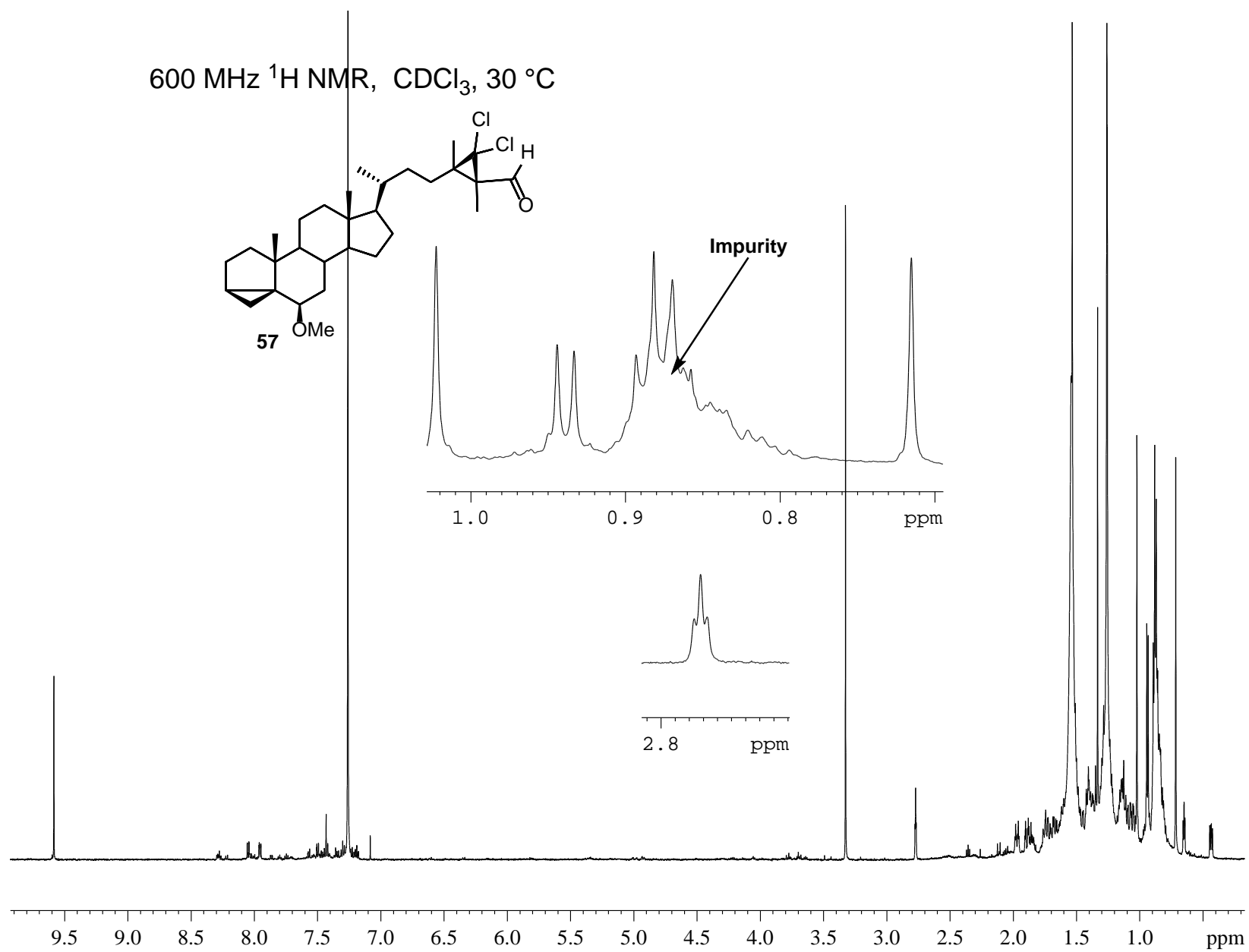


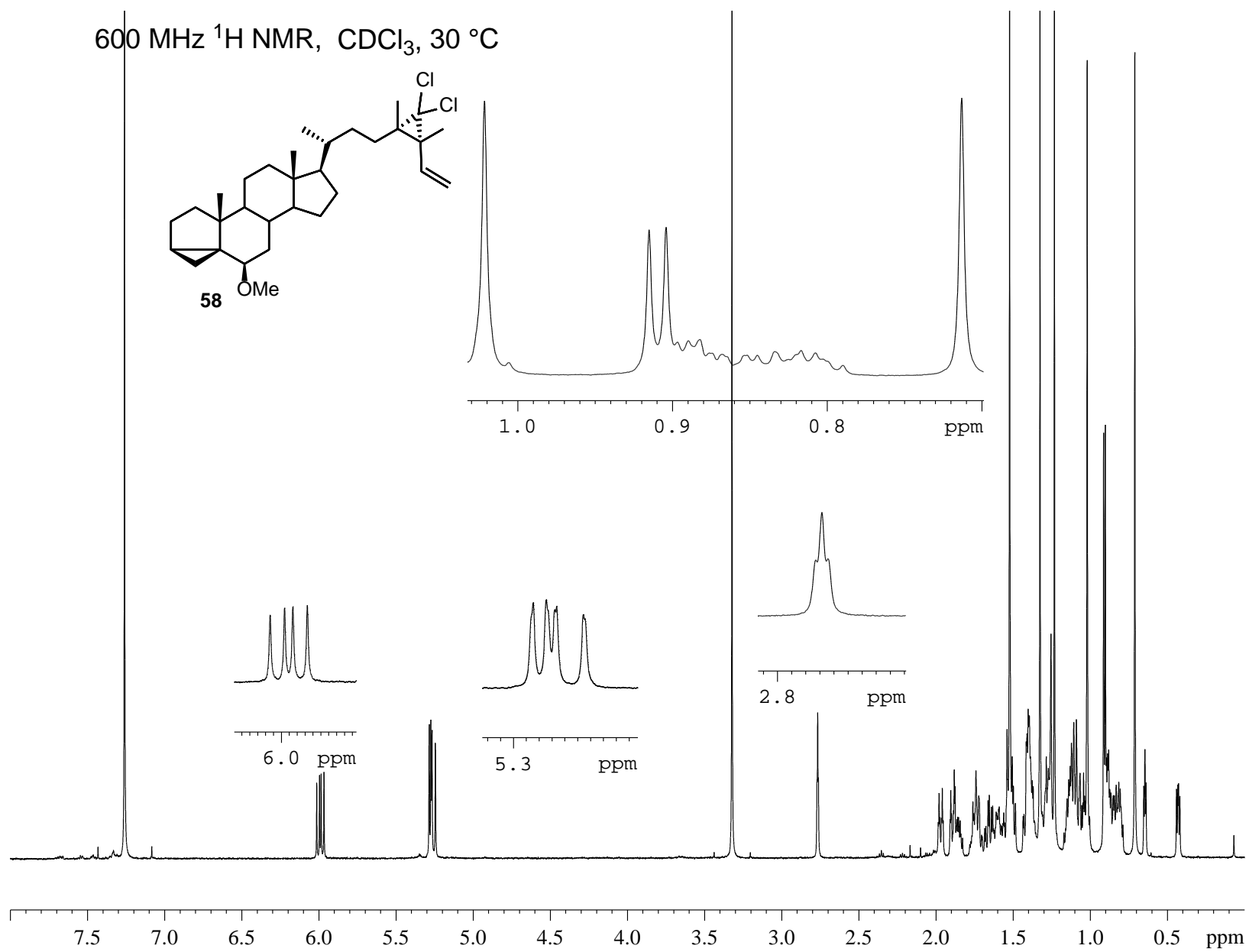


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

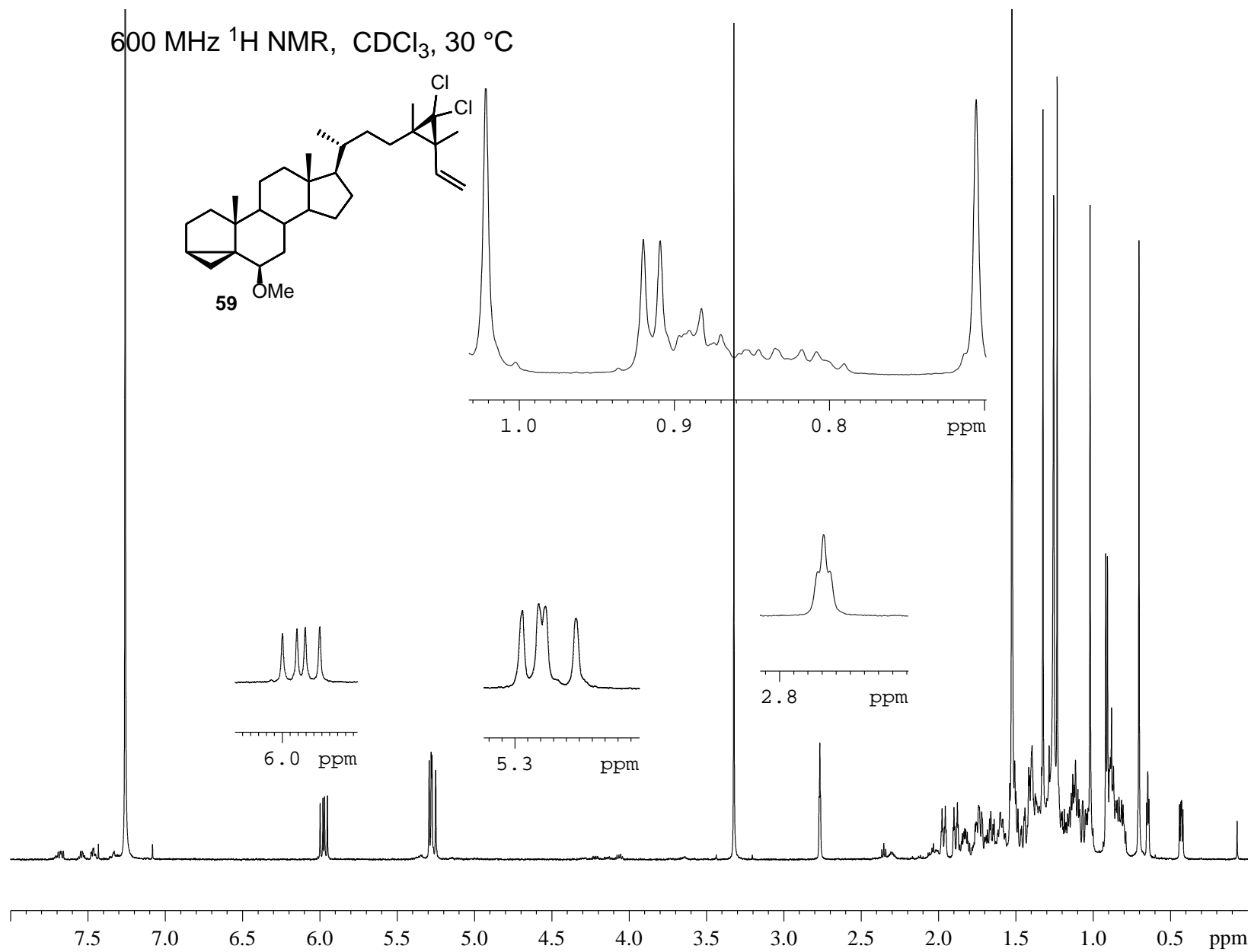
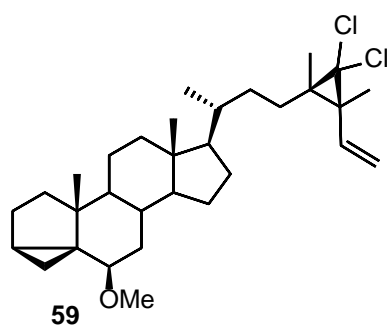


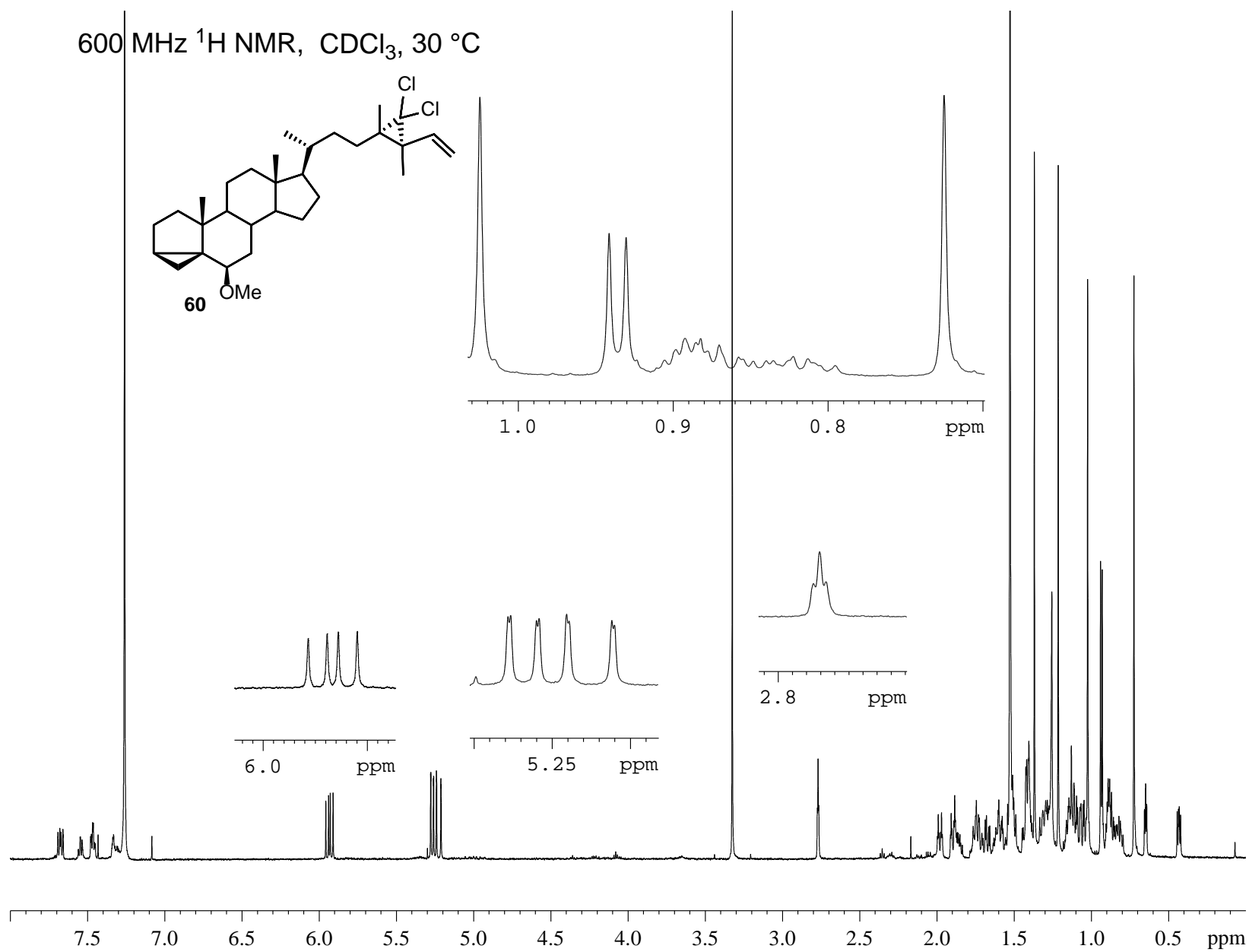


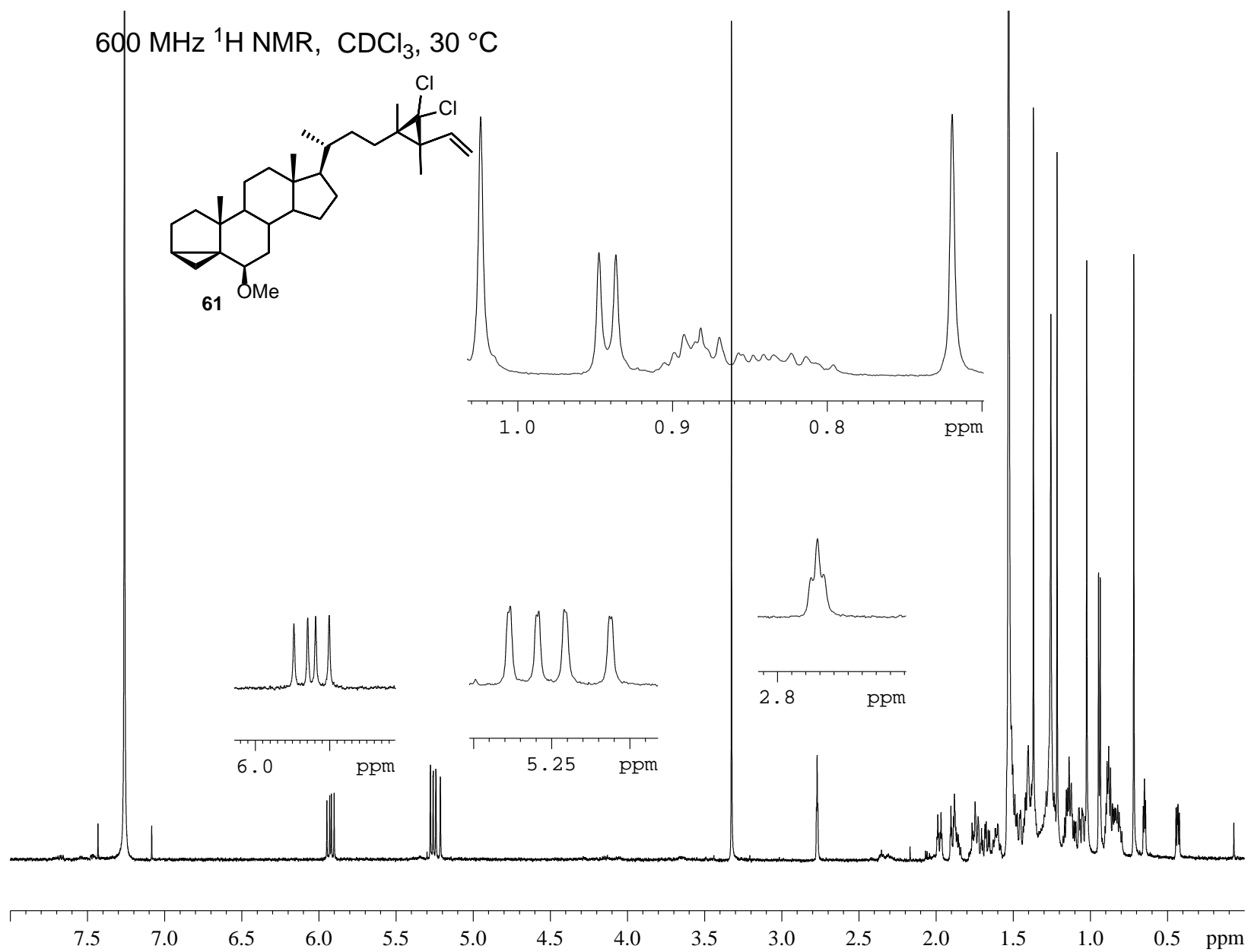


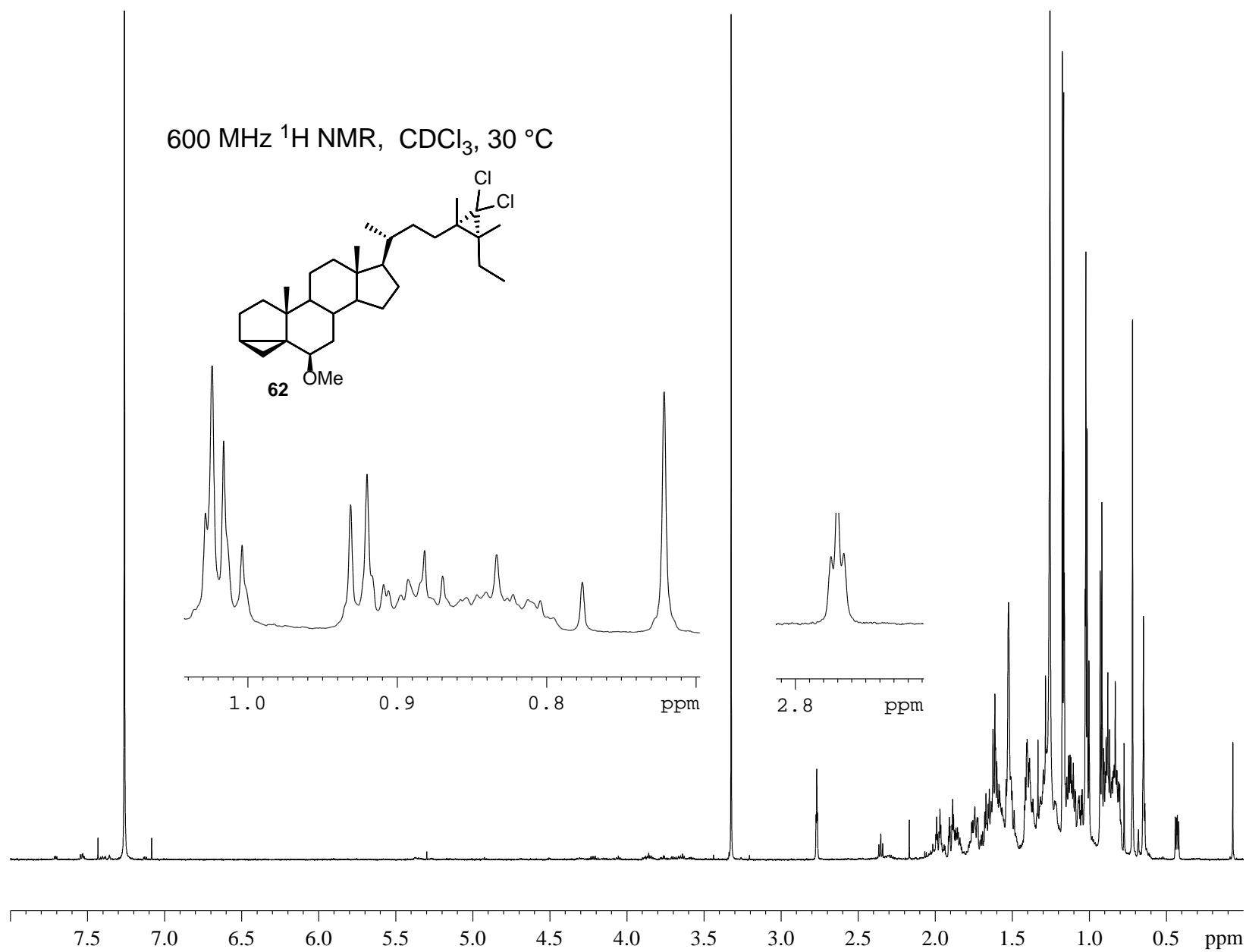


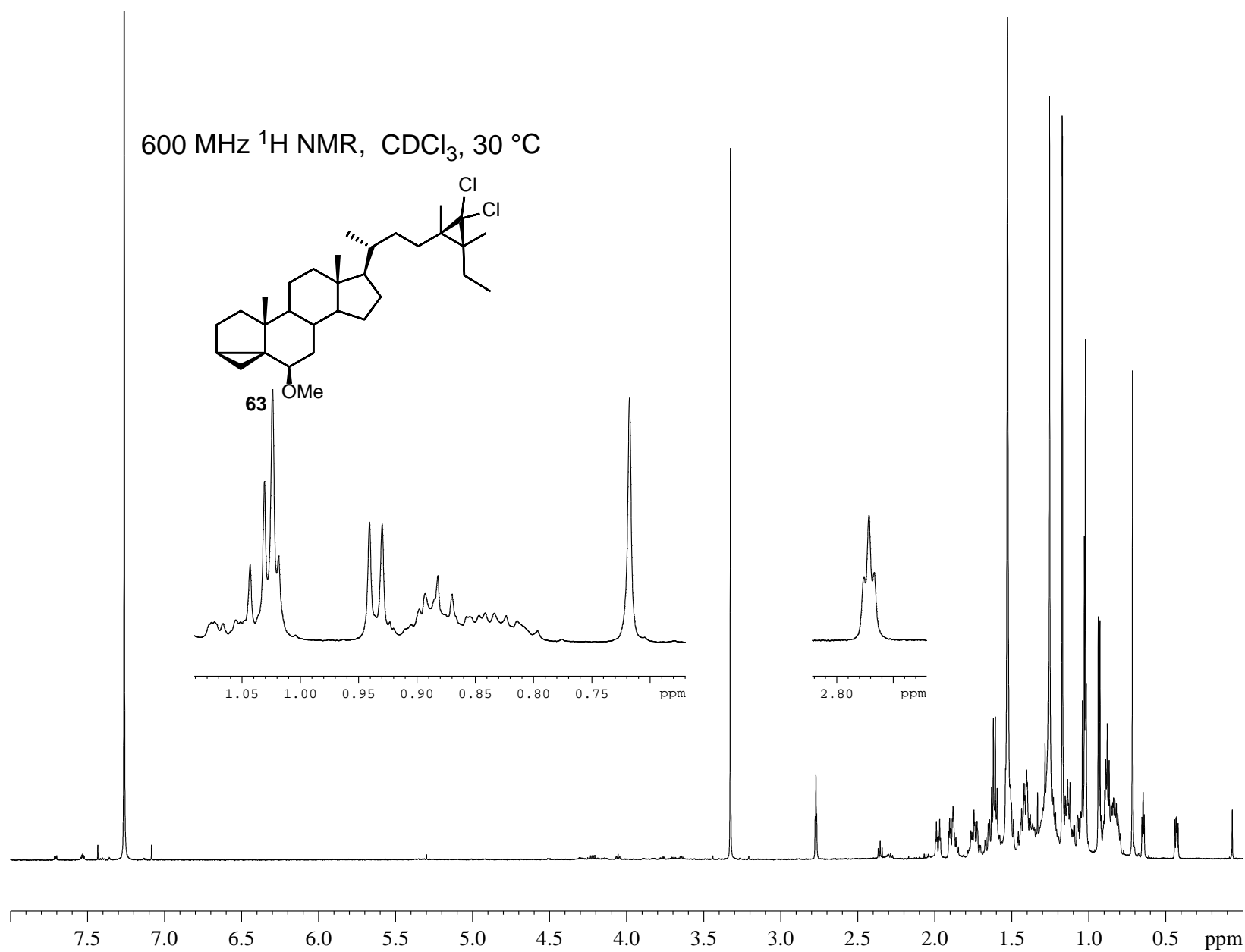
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

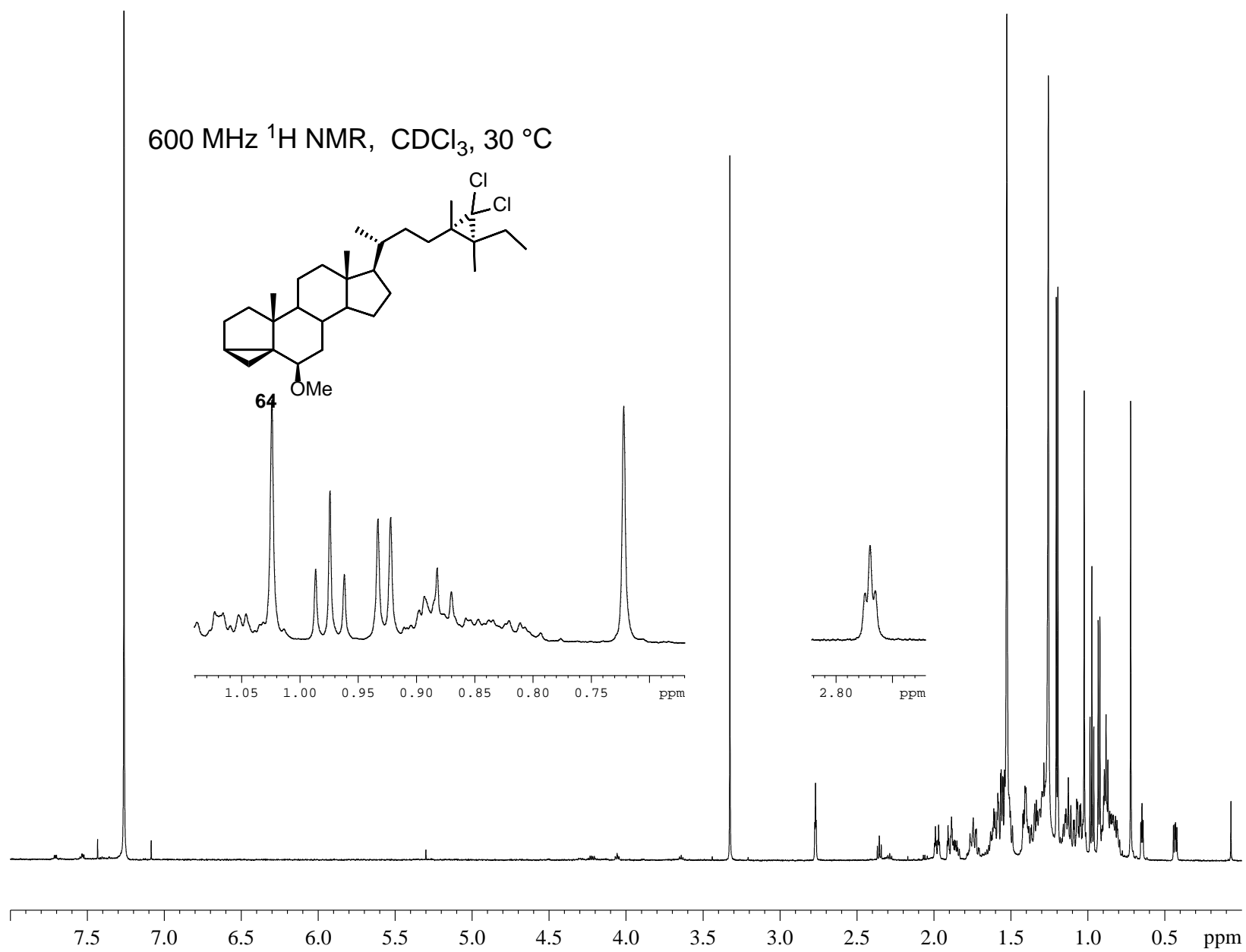




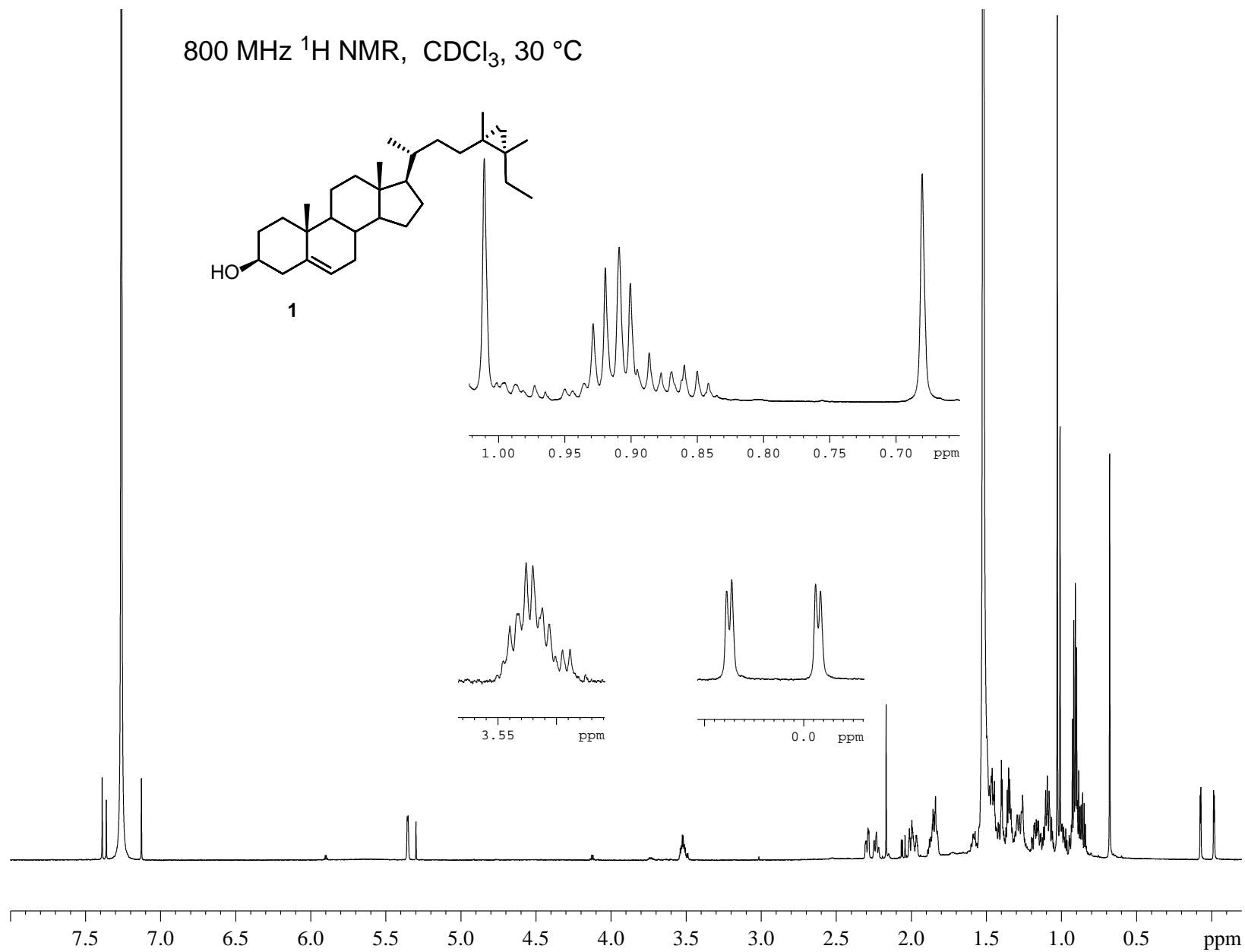
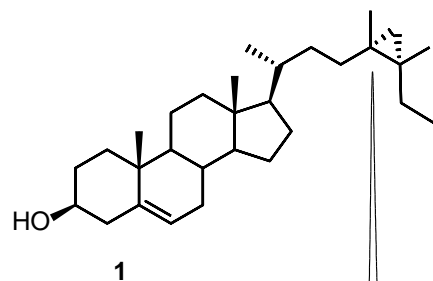




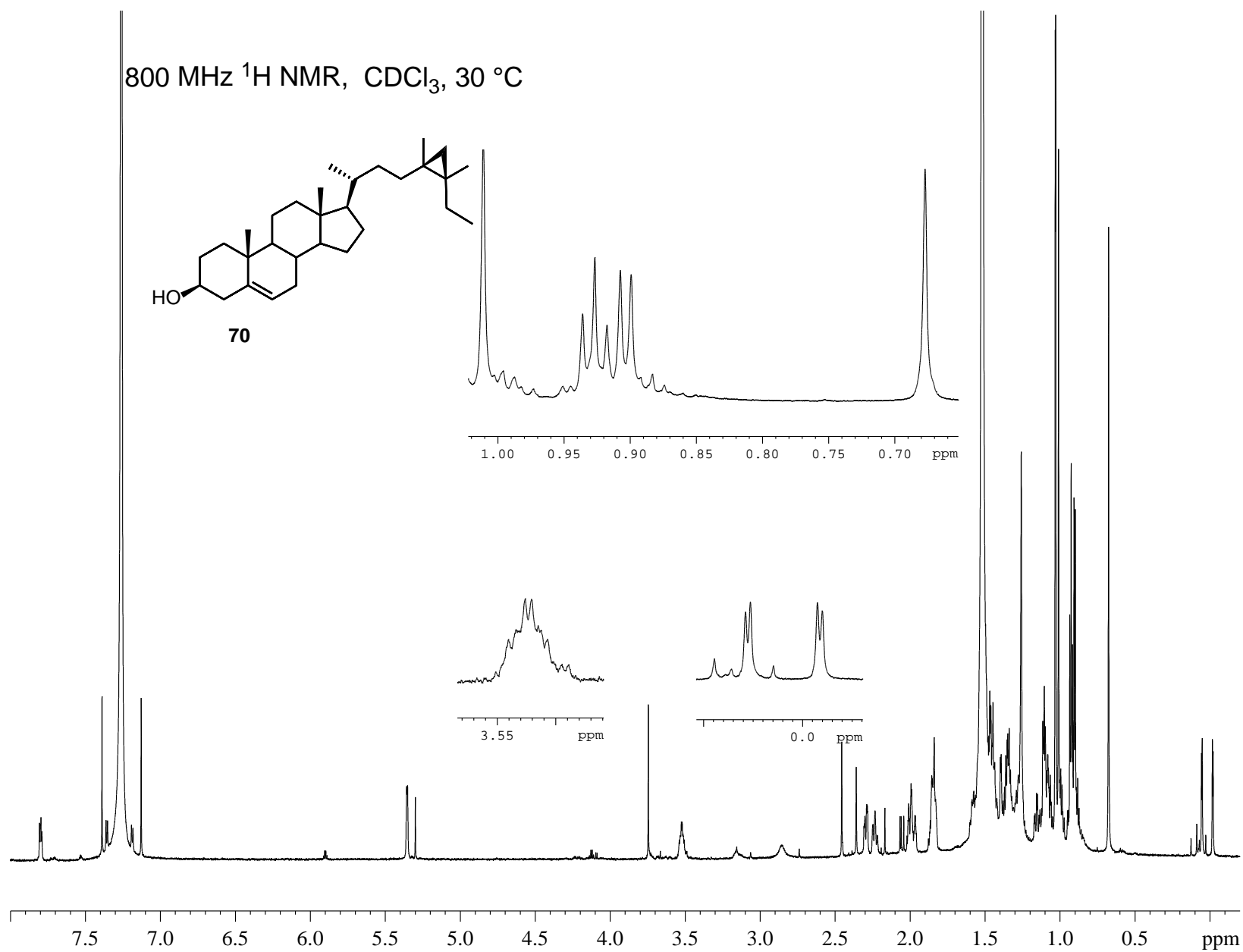




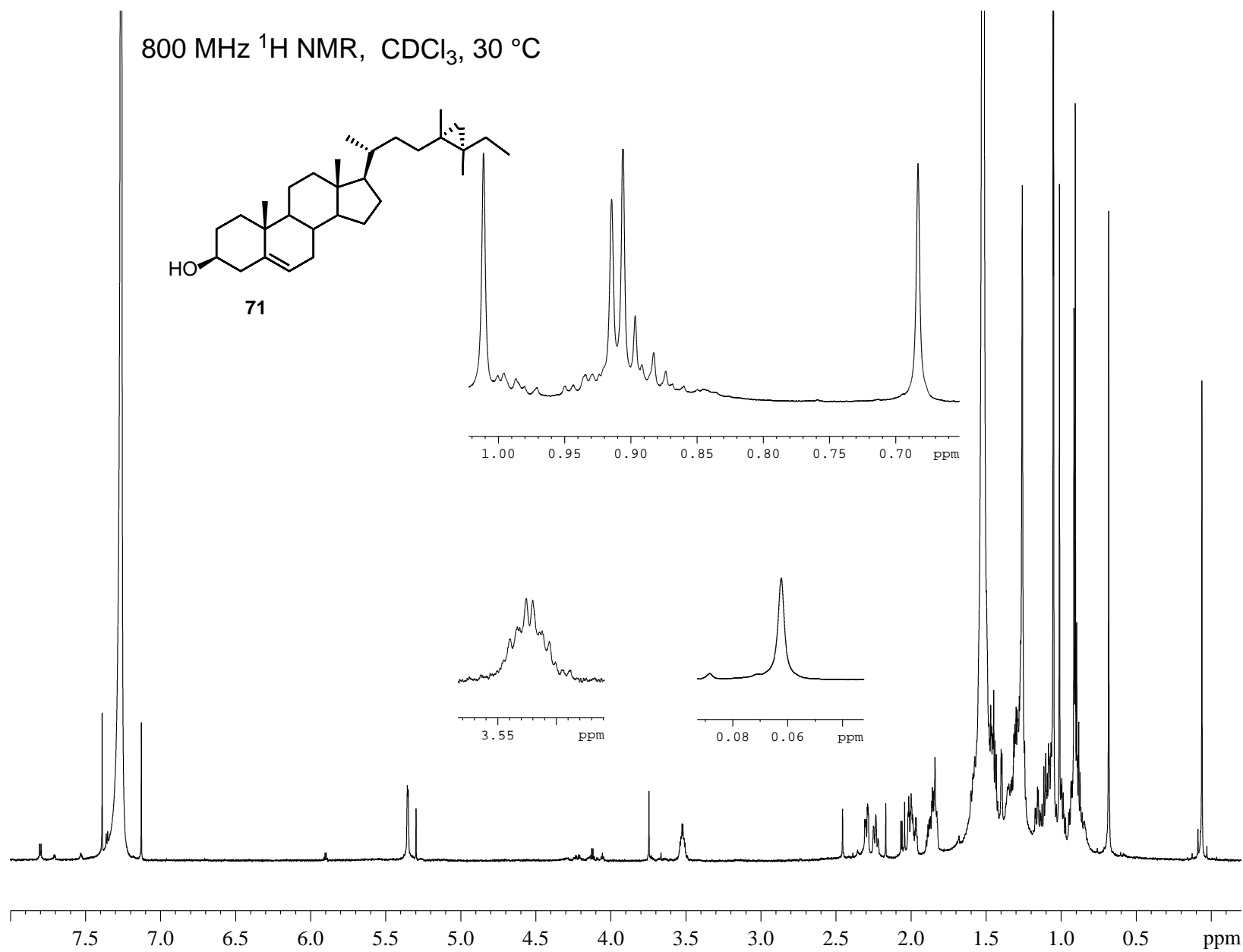
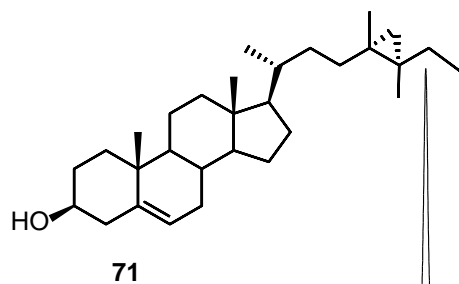
800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



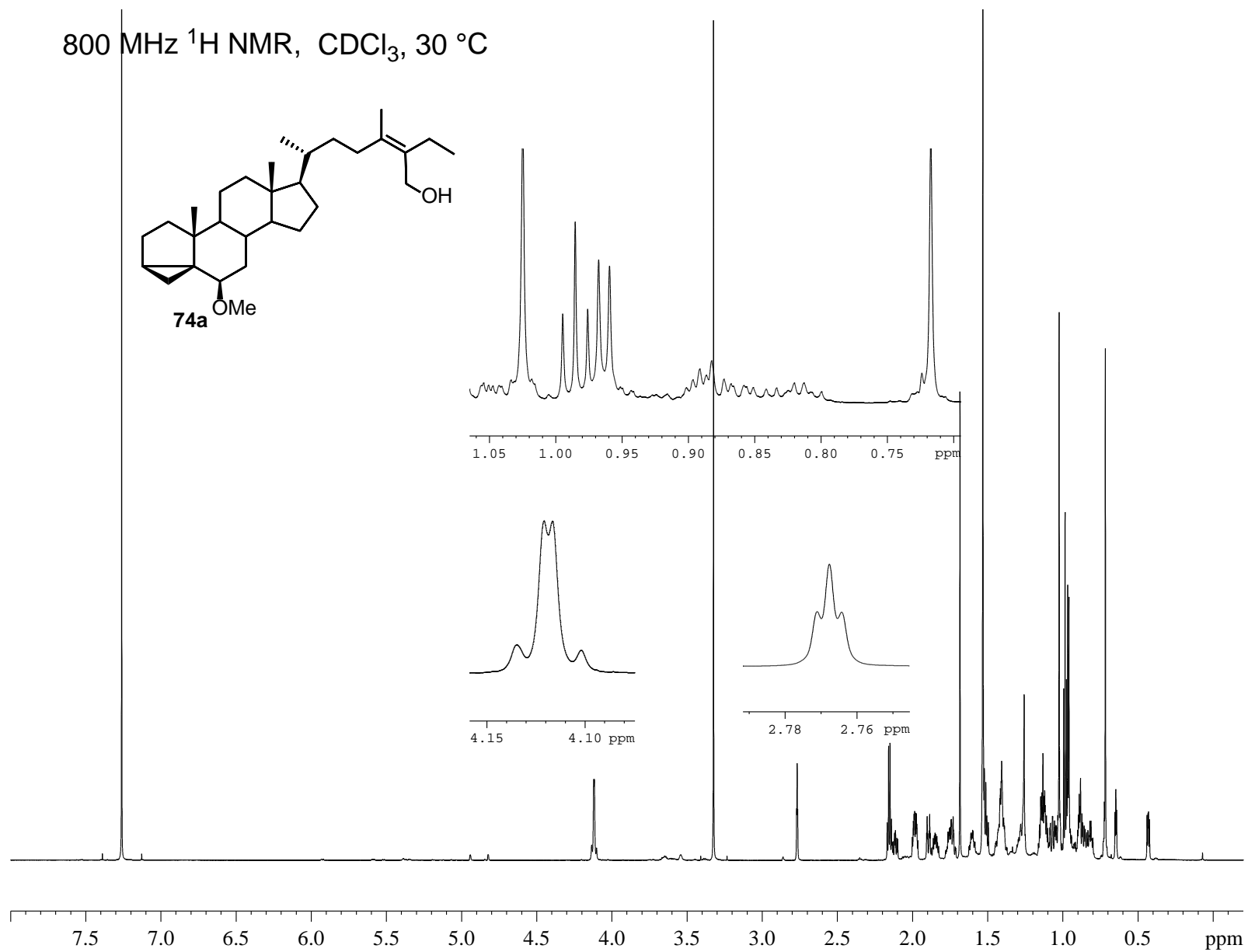
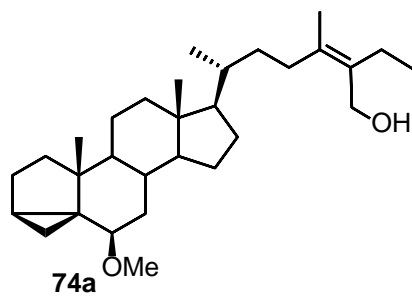
800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

72

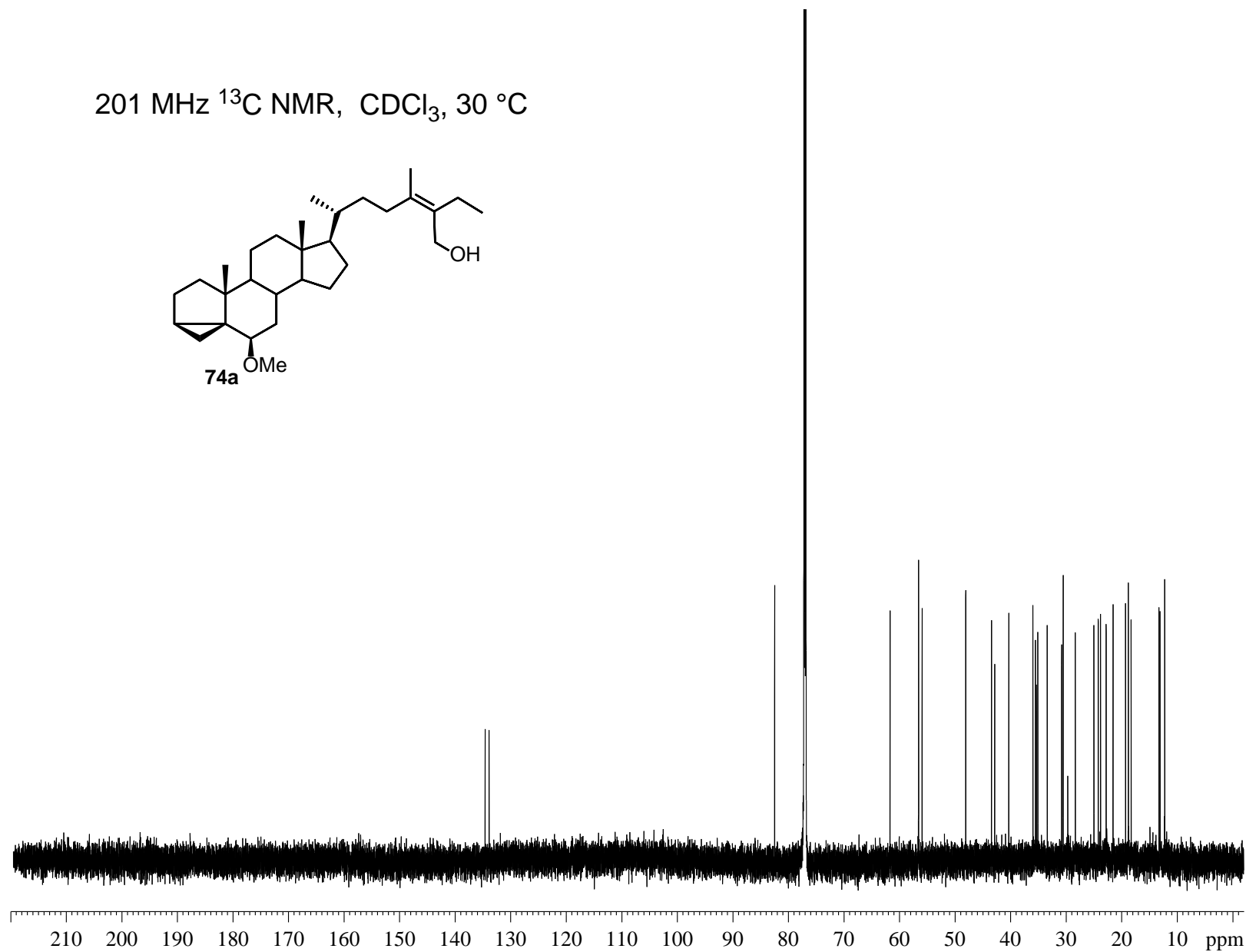
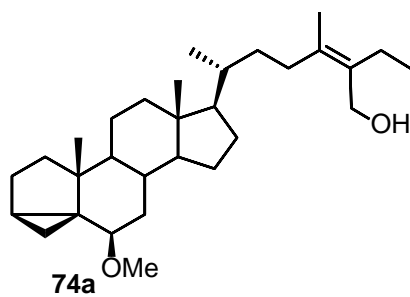
The chemical structure of **72** is a steroid derivative with a hydroxyl group (HO-) and a complex side chain. The NMR spectrum displays the following features:

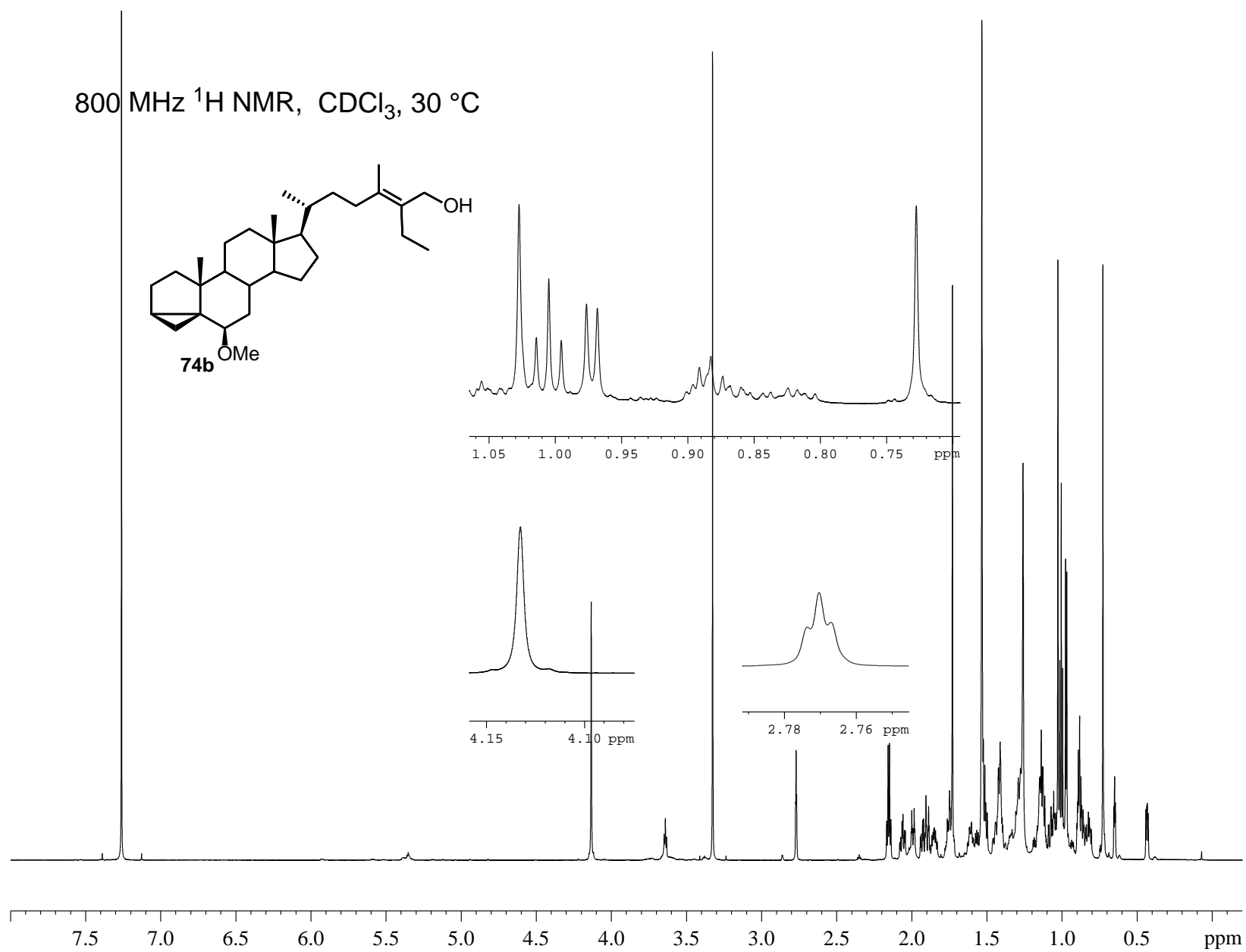
- Chemical Shift Range:** 0 to 7.5 ppm.
- Key Peaks:**
 - Aromatic region (7.0-7.5 ppm): Two sharp singlets.
 - Aliphatic region (0.5-2.5 ppm): Multiple multiplets and singlets, including a prominent peak around 1.0 ppm.
- Insets:**
 - Inset 1 (3.55-4.0 ppm):** Shows a complex multiplet pattern.
 - Inset 2 (2.9-3.1 ppm):** Shows a complex multiplet pattern.

800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

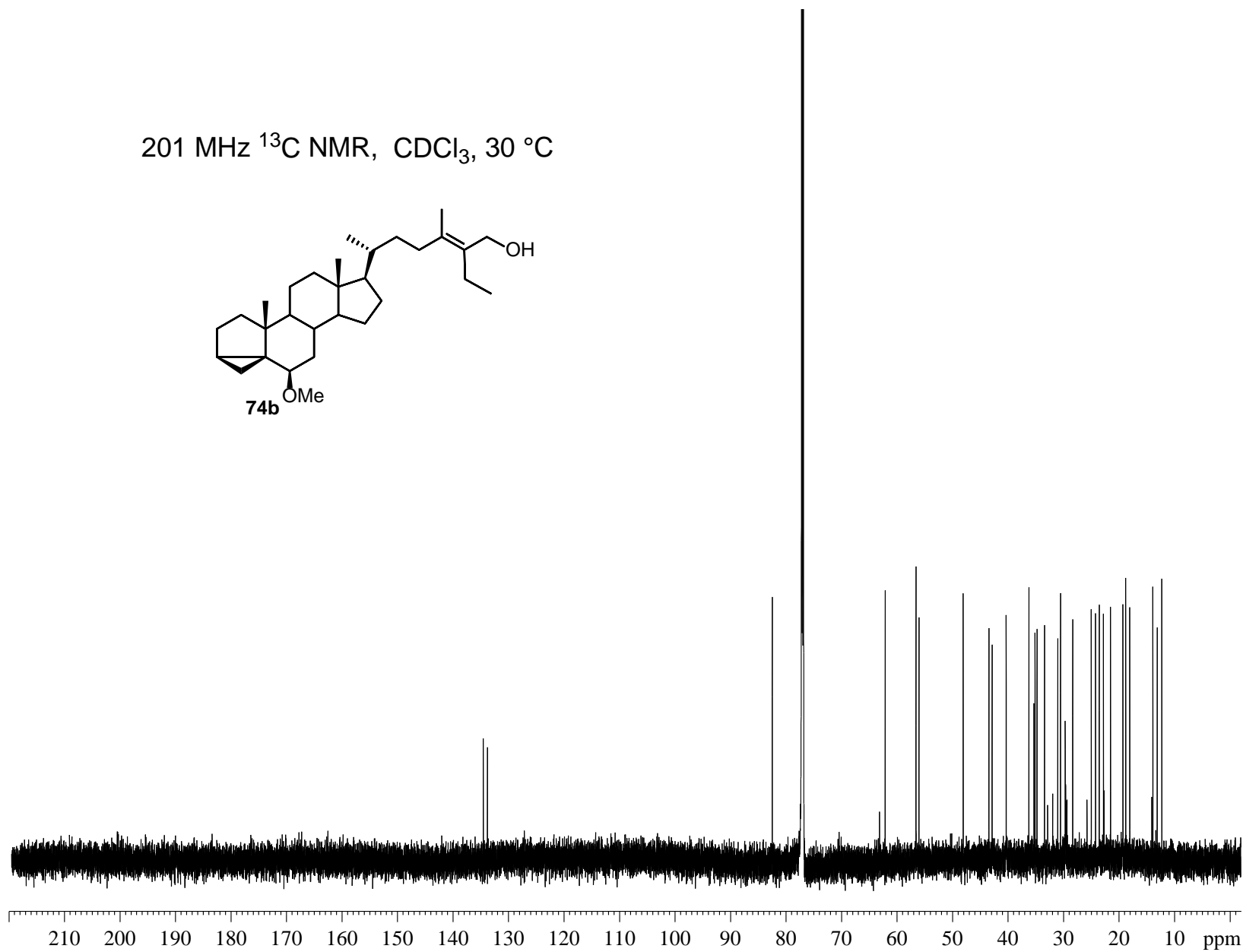
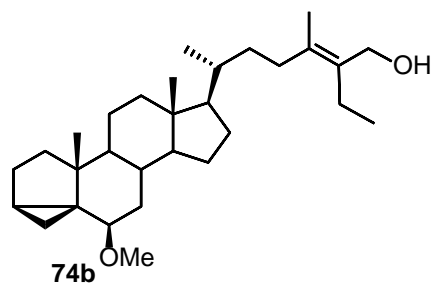


201 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$





201 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

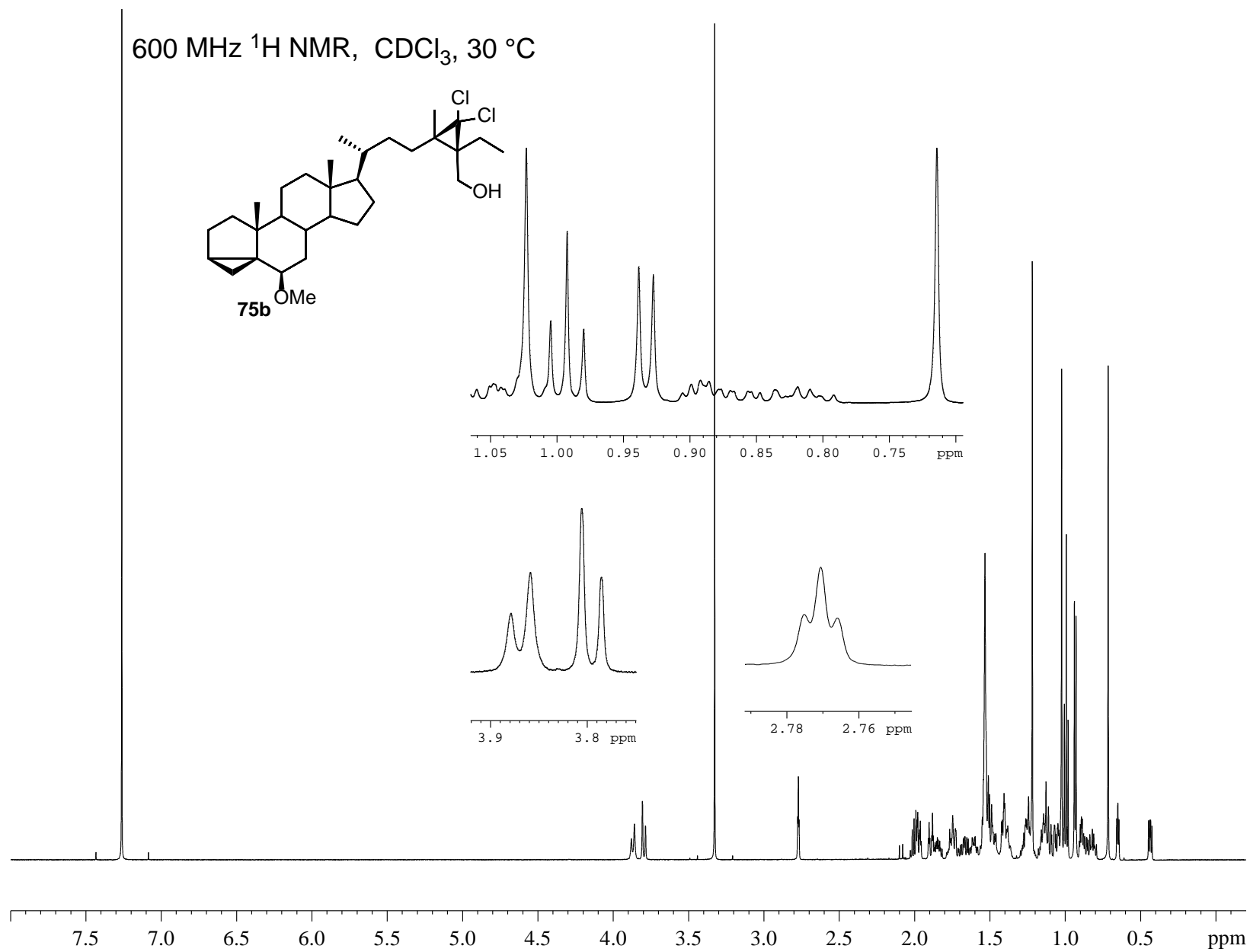
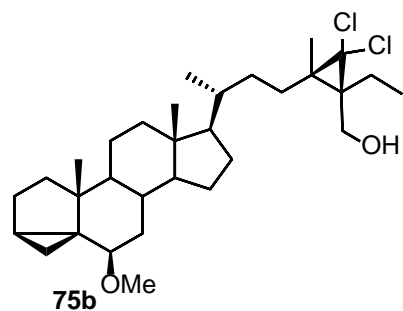
75a

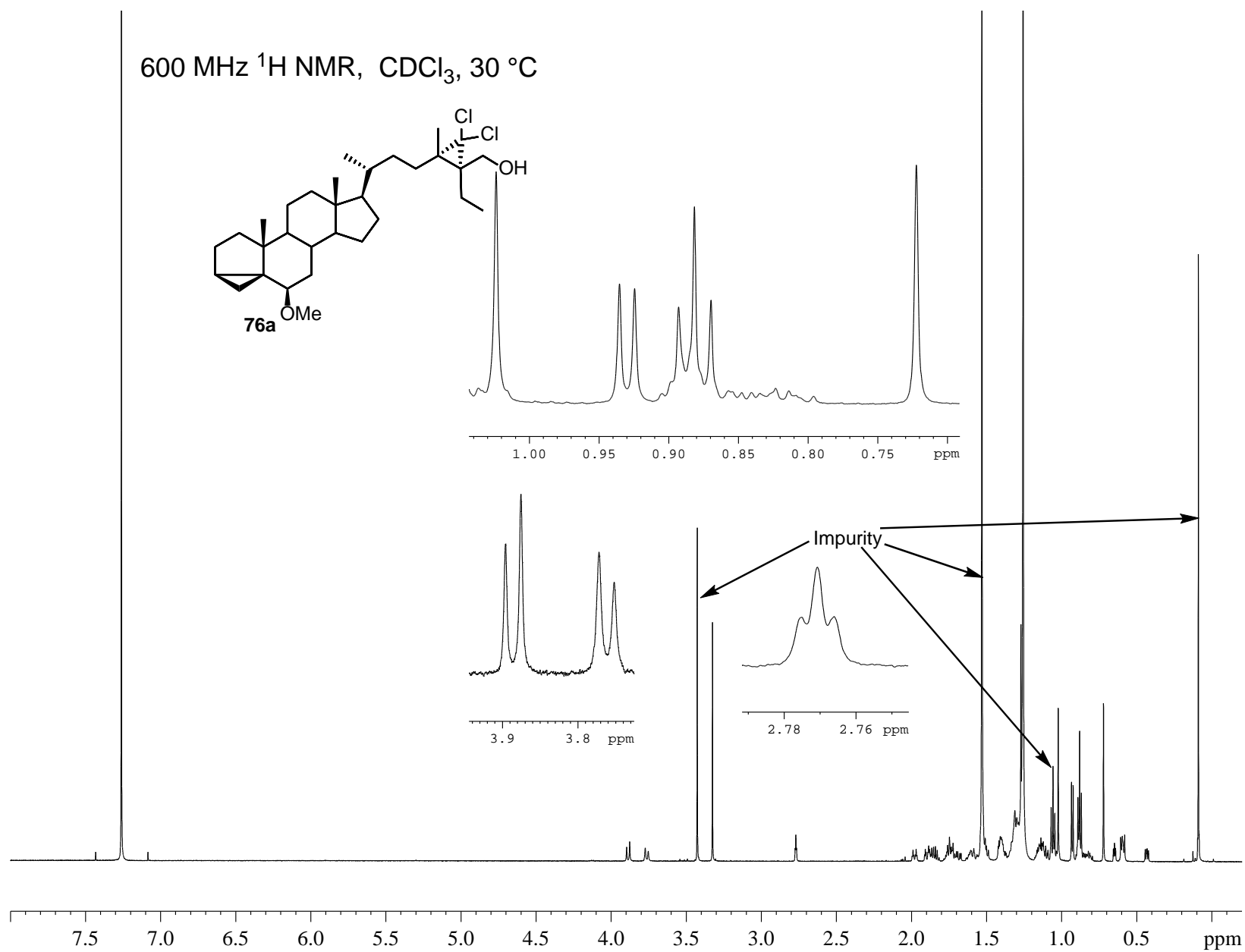
Chemical structure of **75a** is shown as an inset. The structure is a steroid derivative with a methoxy group (OMe) and a side chain containing two chlorine atoms (Cl) and a hydroxyl group (OH).

The NMR spectrum displays peaks in the following regions (ppm):

- Aliphatic region (0.5 - 2.5 ppm): Multiple peaks, including a prominent peak at approximately 1.0 ppm and a cluster of peaks between 0.5 and 2.0 ppm.
- Region 0.90 - 1.05 ppm: Expanded view showing several distinct peaks.
- Region 2.76 - 2.78 ppm: Expanded view showing a single peak.
- Region 3.9 ppm: A small peak.

600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$





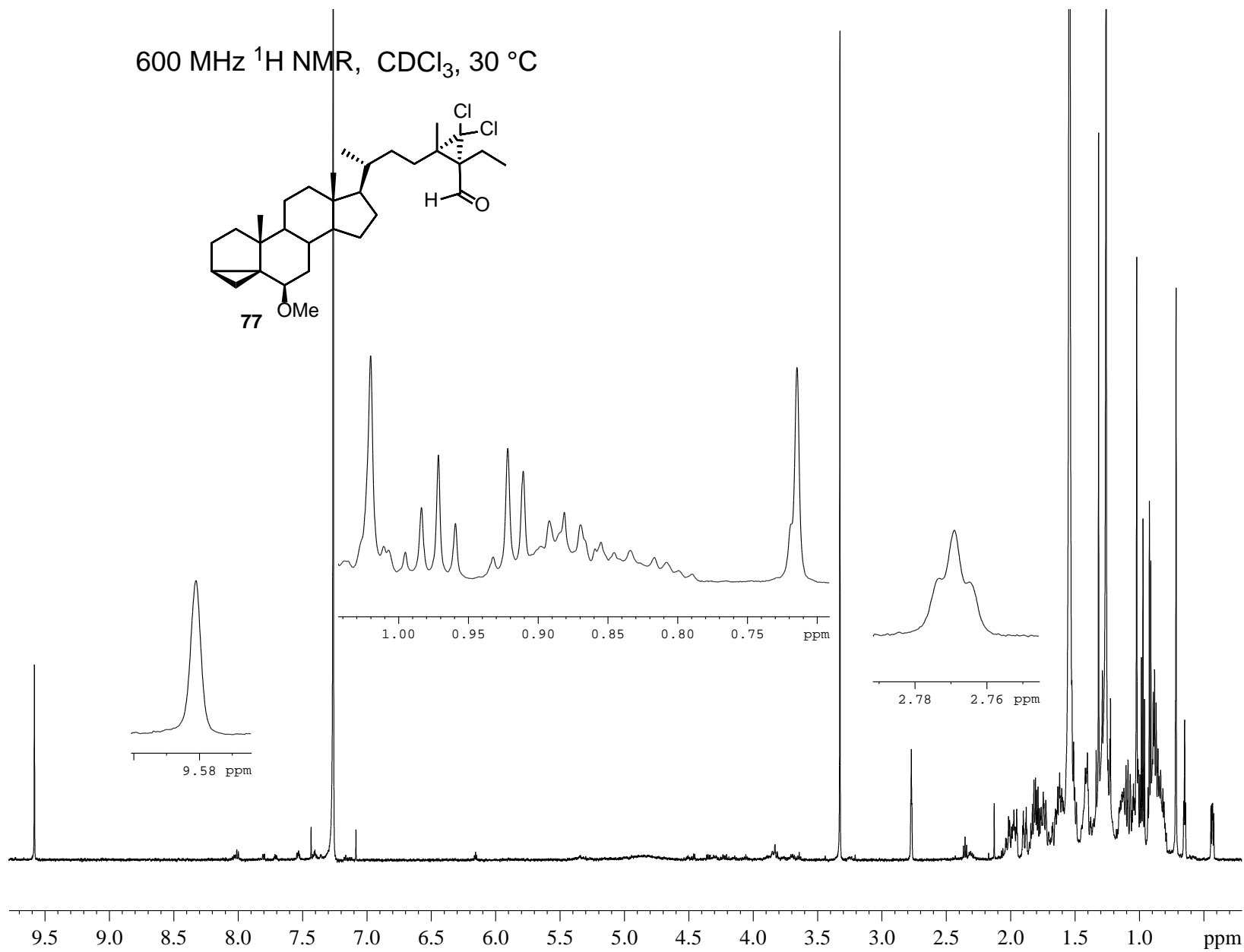
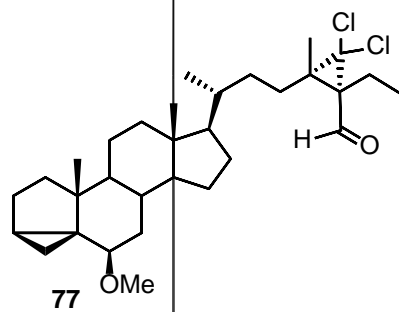
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

76b

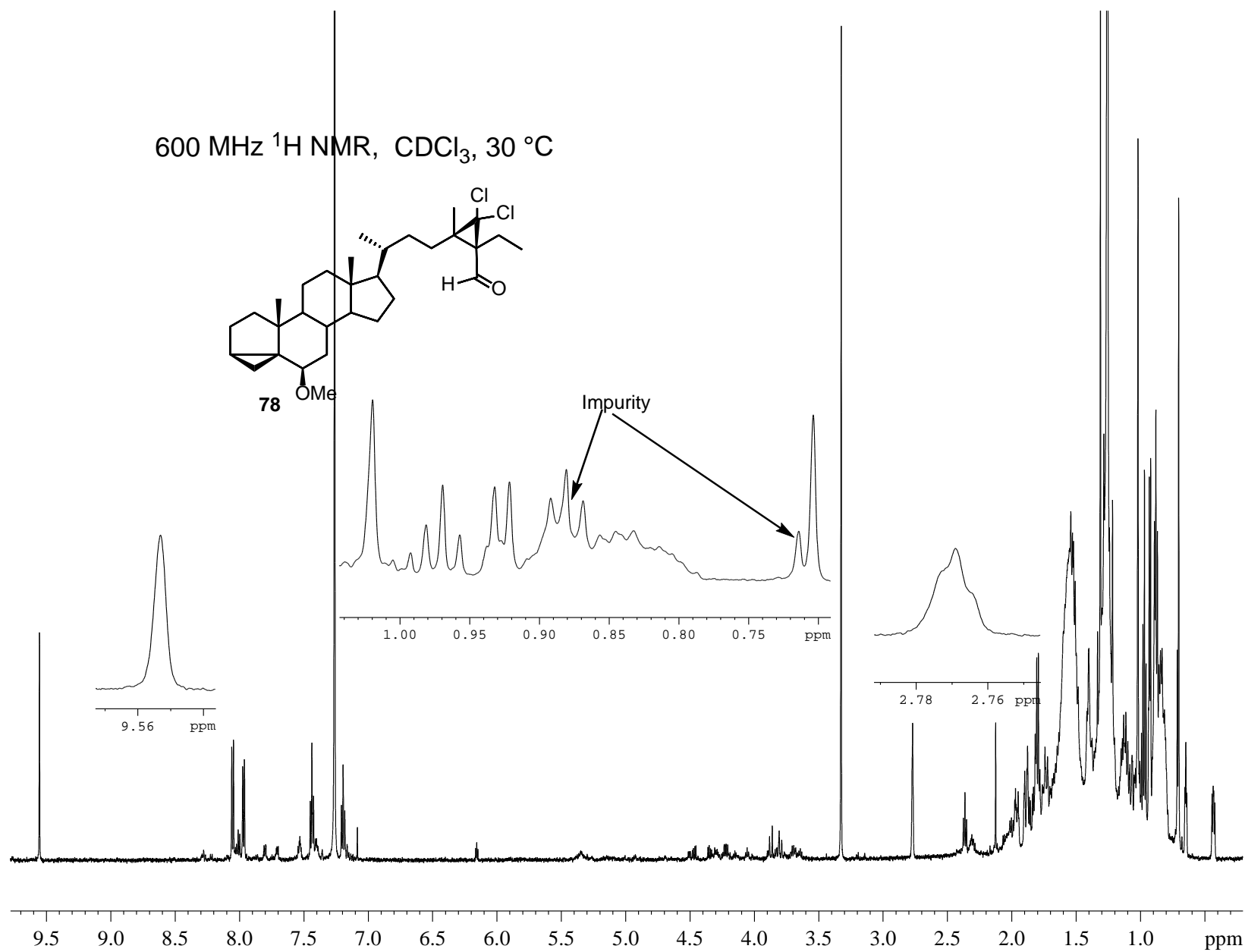
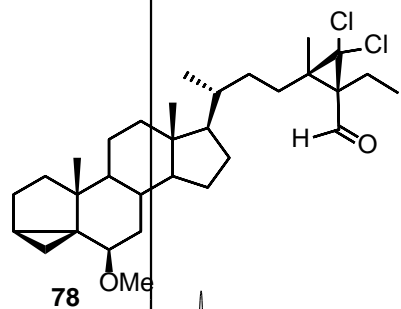
The chemical structure of **76b** is shown, featuring a steroid-like core with a methoxy group (OMe) and a side chain containing two chlorine atoms (Cl) and a hydroxyl group (OH).

The NMR spectrum displays several peaks, with a prominent peak at approximately 3.4 ppm. An inset shows a zoomed-in view of the region around 3.8 ppm, and another inset shows a zoomed-in view of the region around 2.76 ppm, with an arrow pointing to a peak labeled "Impurity".

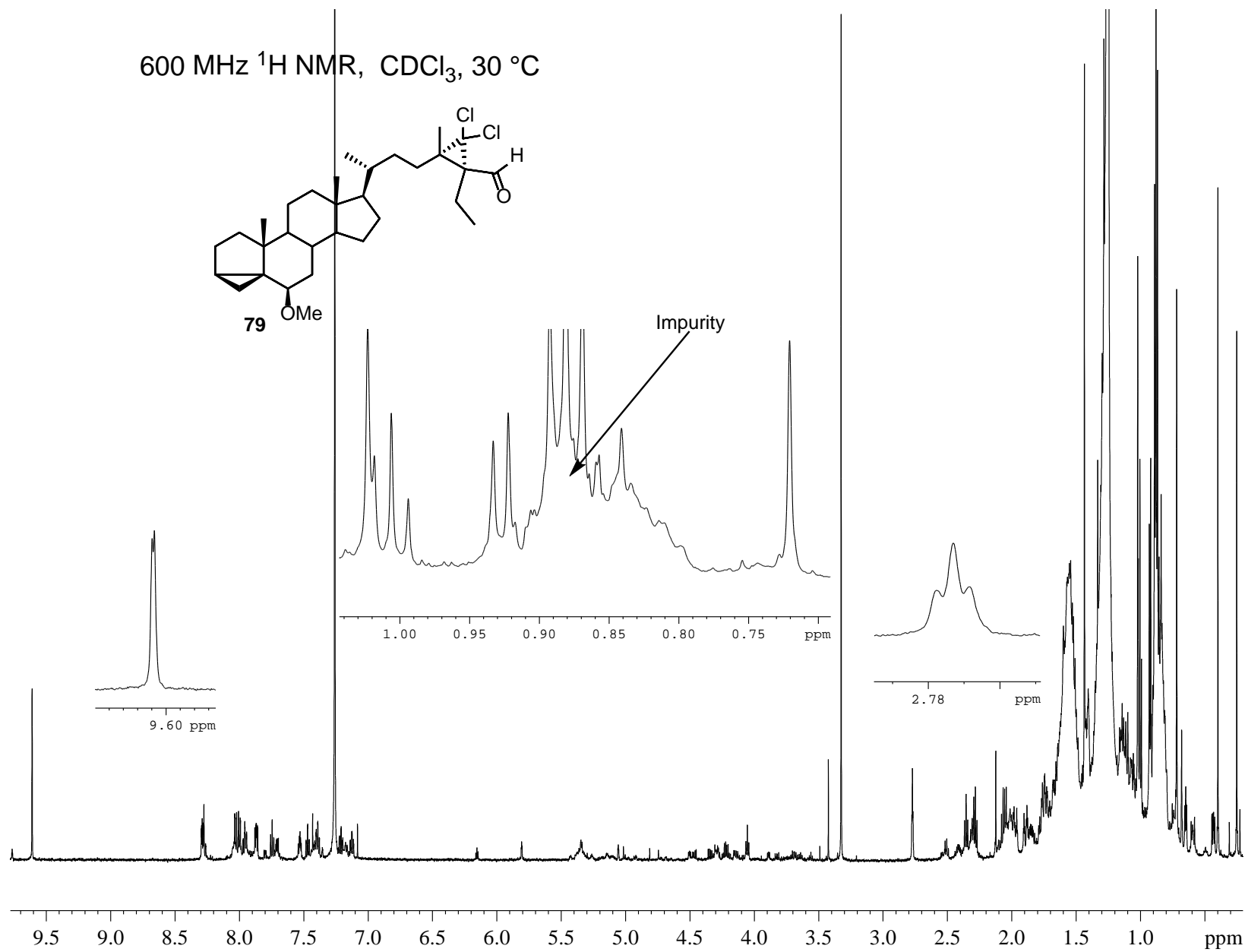
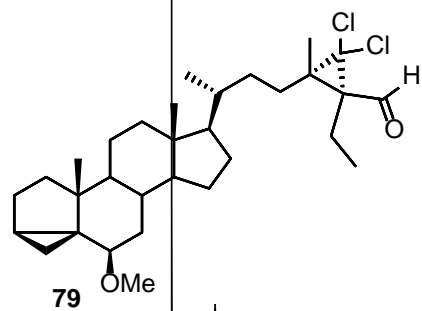
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



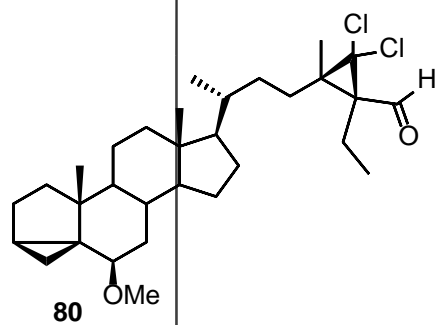
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



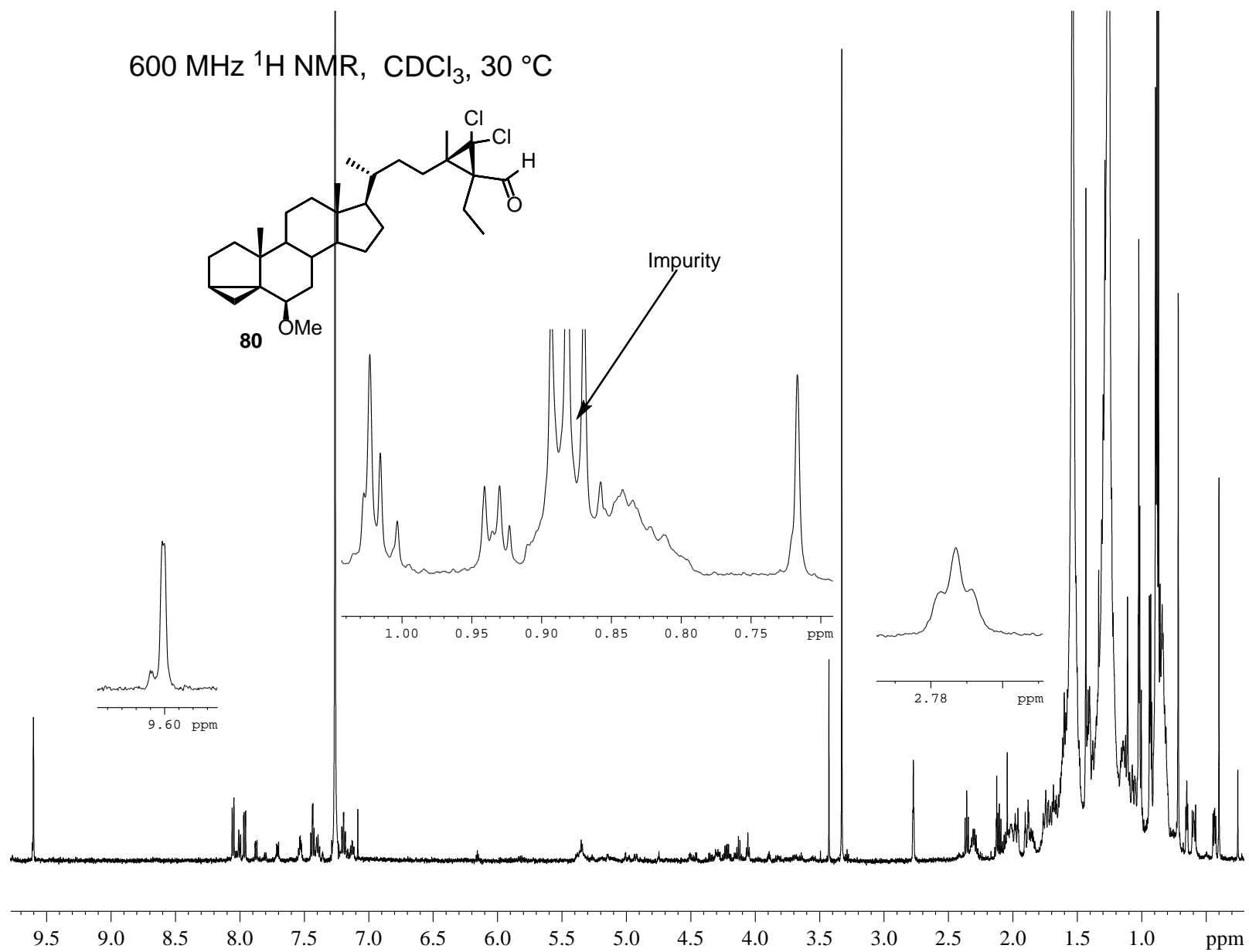
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



Impurity



600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

81

OMe

Impurity

2.78 ppm

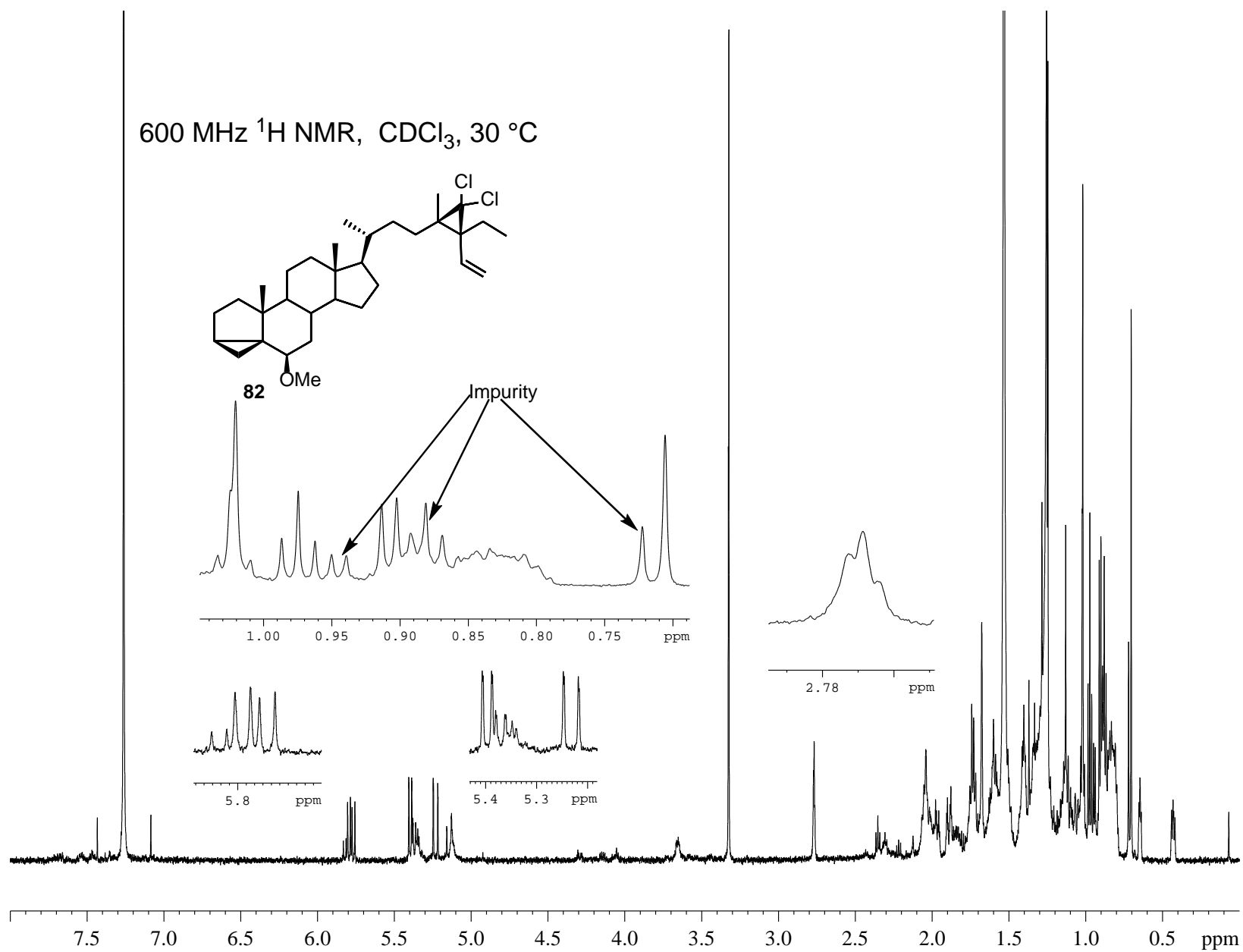
5.8 ppm

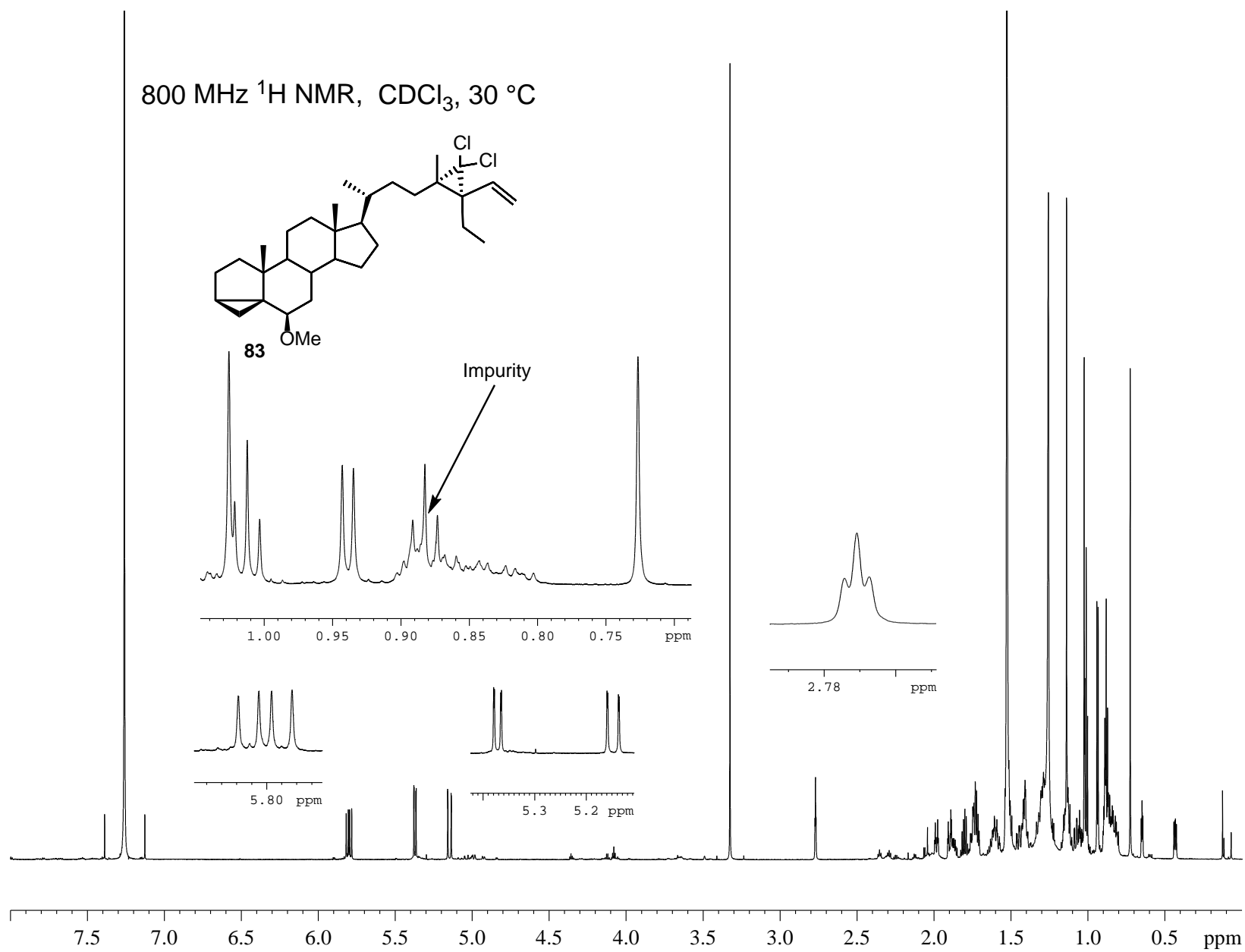
5.4 ppm

5.3 ppm

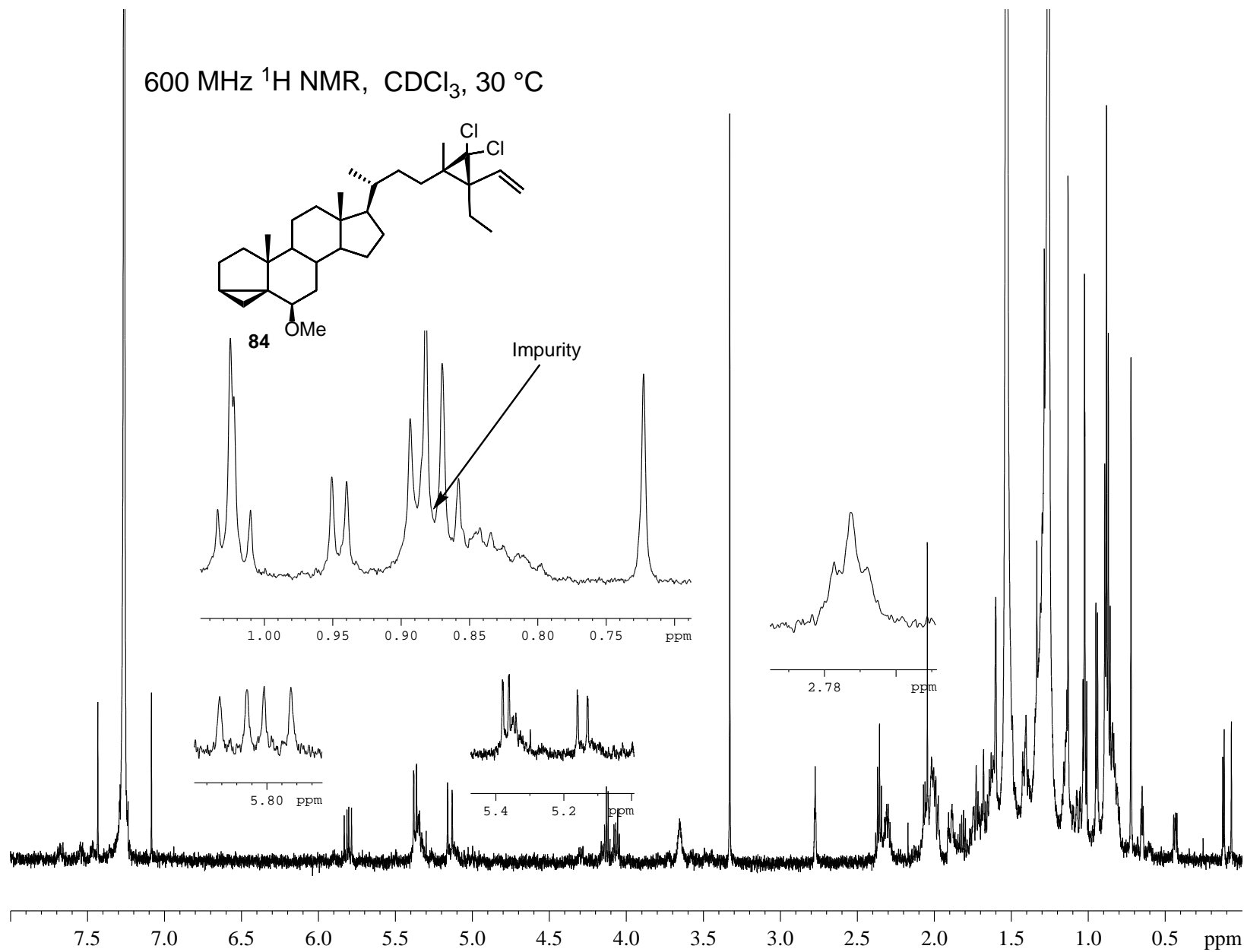
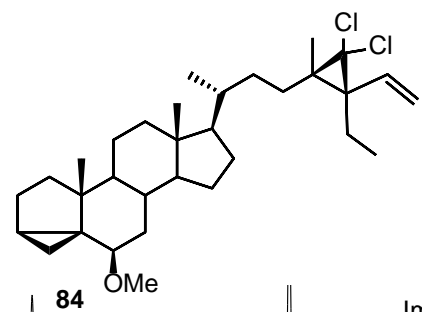
ppm

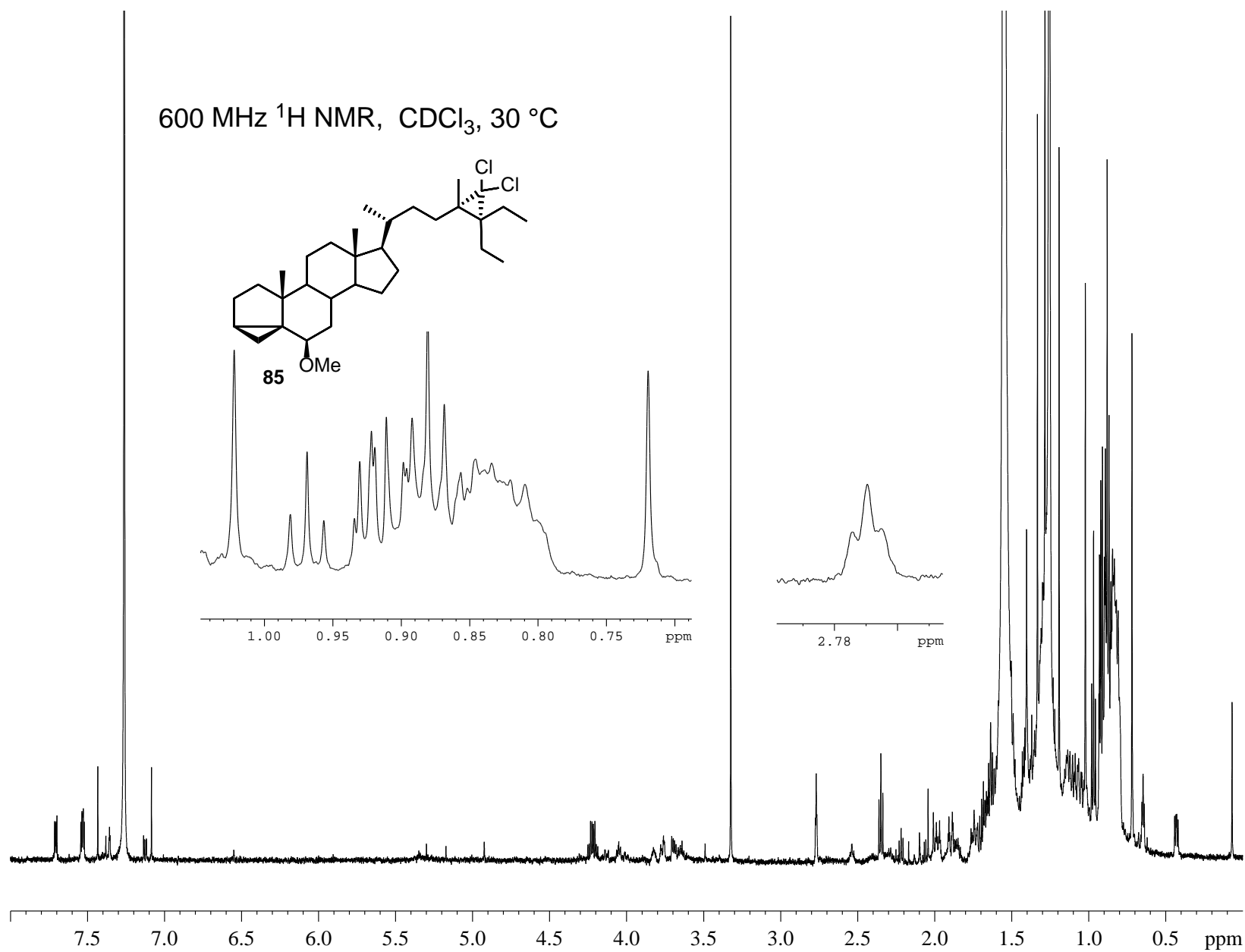
Chemical structure of compound 81 is shown above the spectrum. The structure is a steroid-like molecule with a methoxy group (OMe) and a side chain containing a double bond and two chlorine atoms.



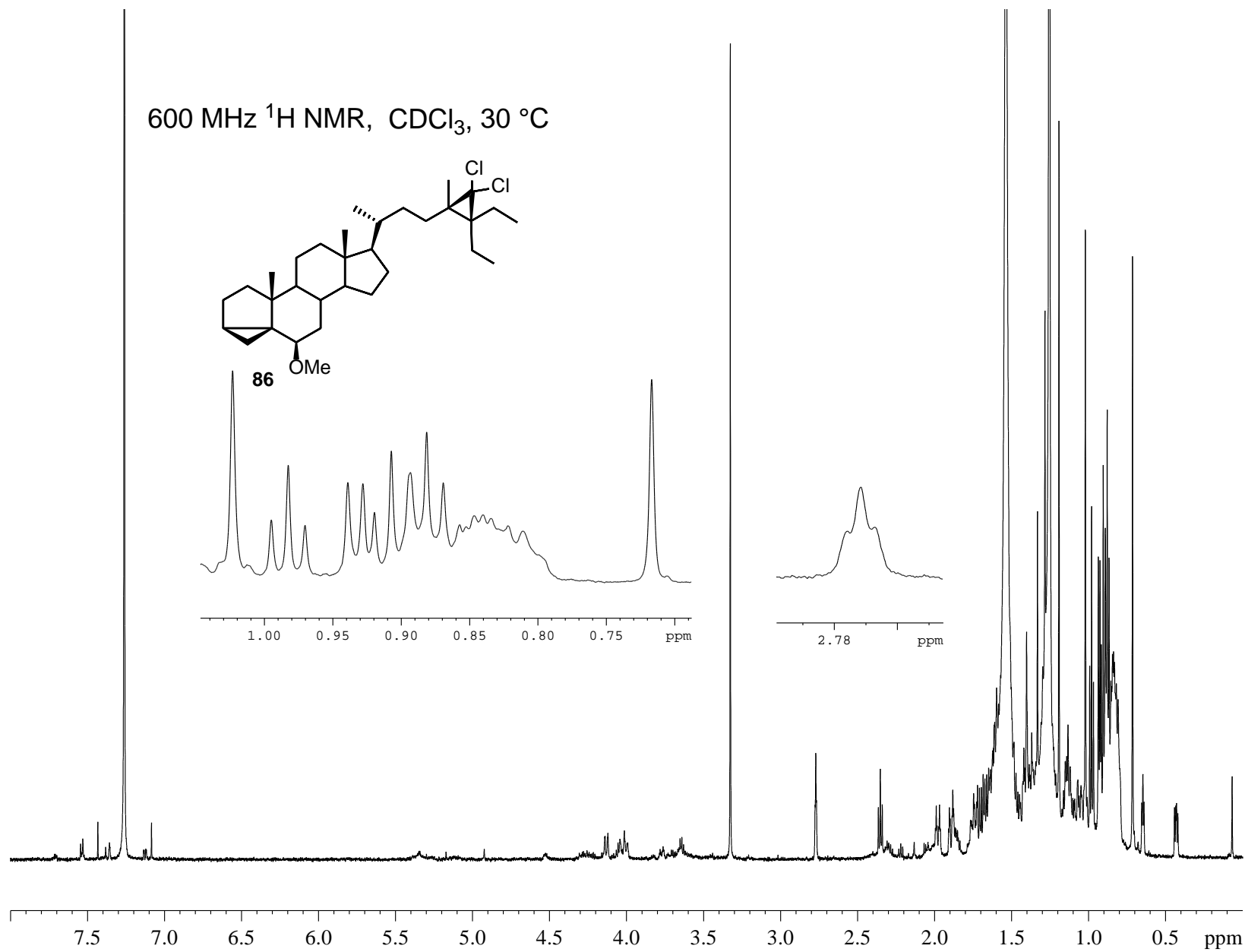
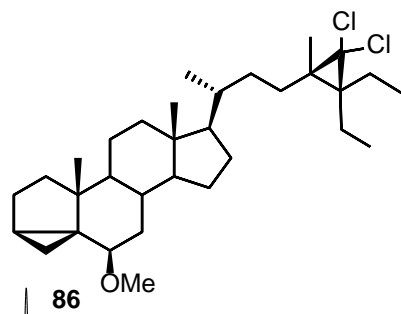


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

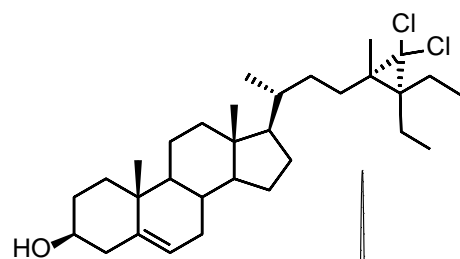




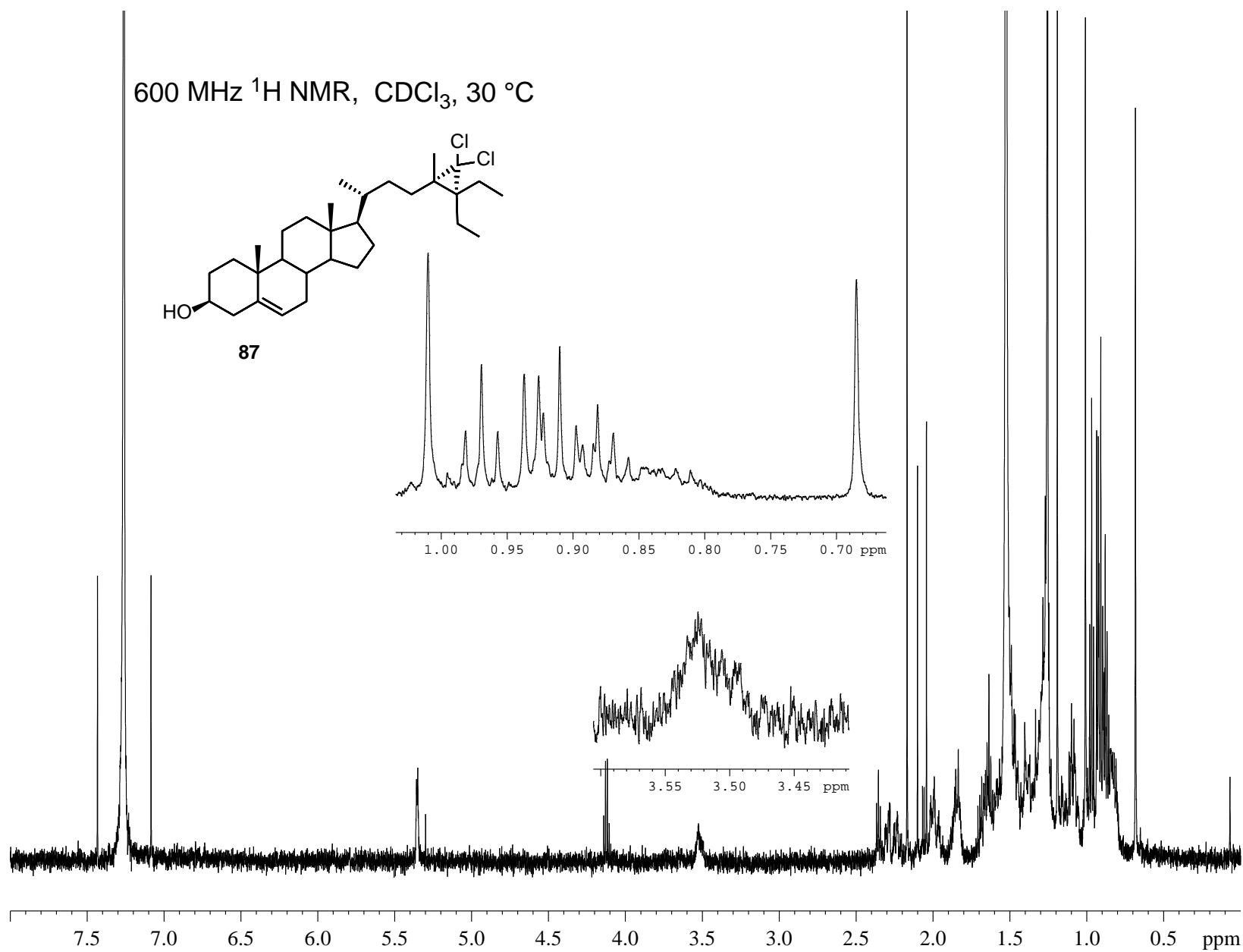
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

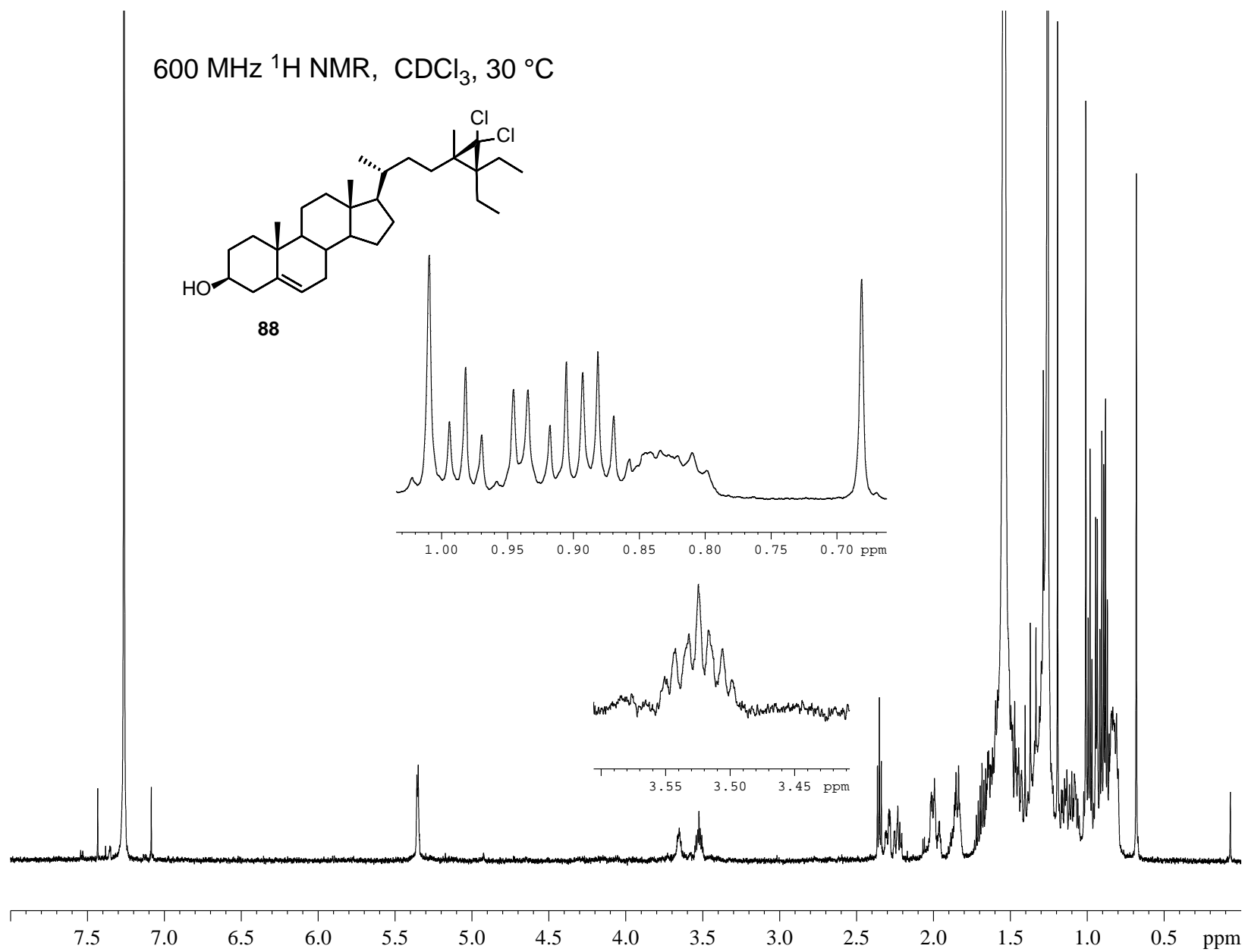


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

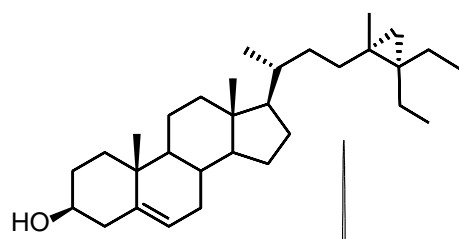


87

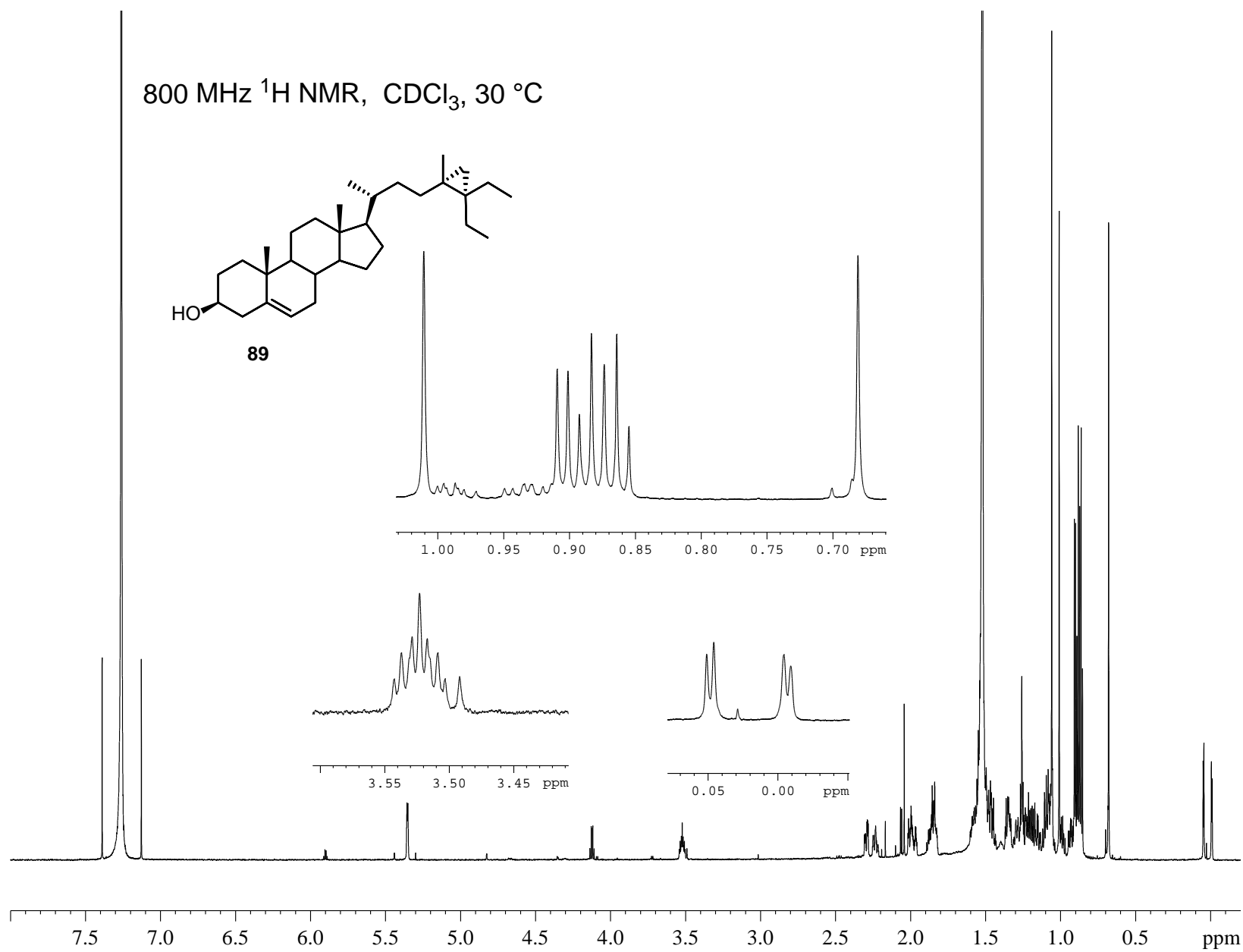


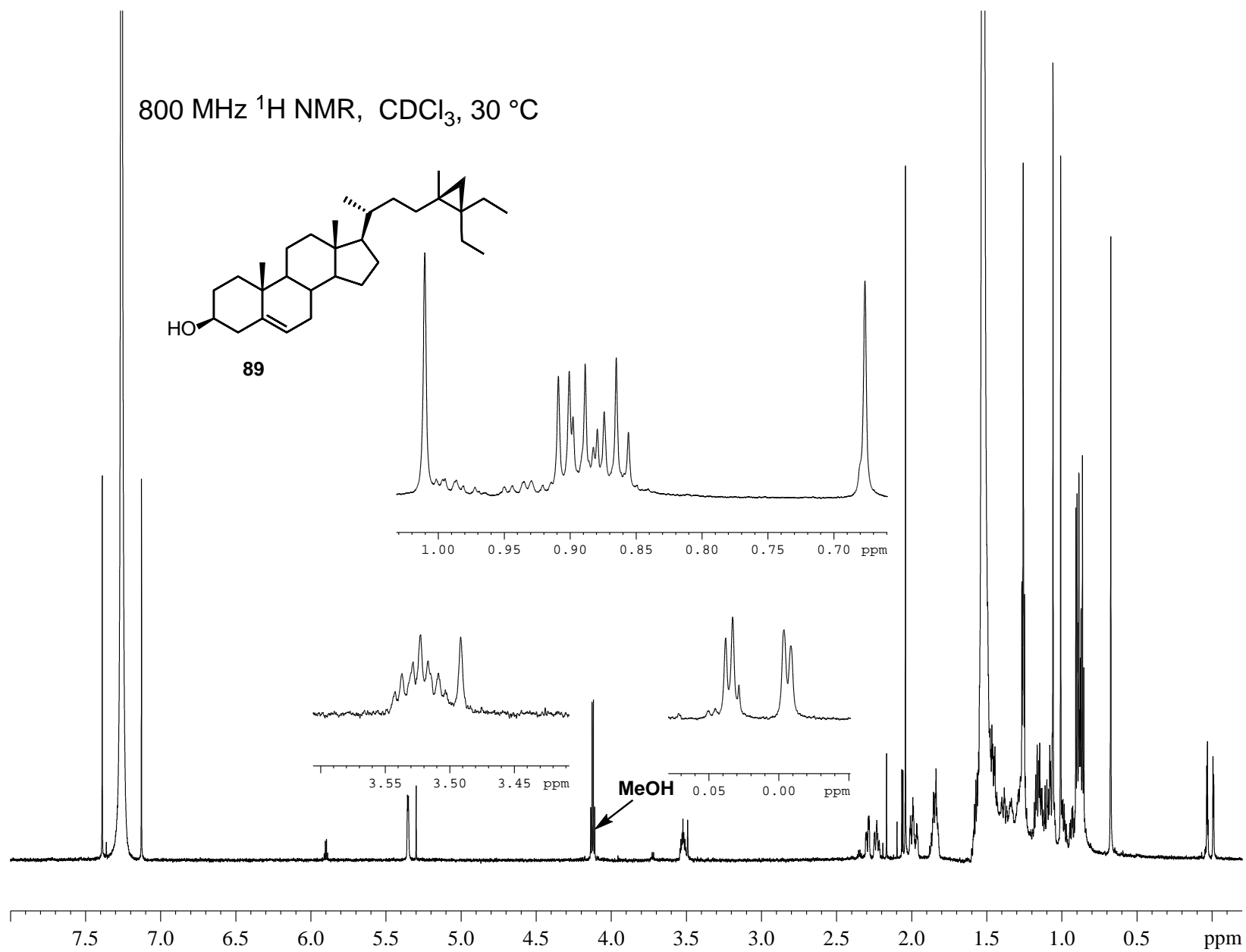


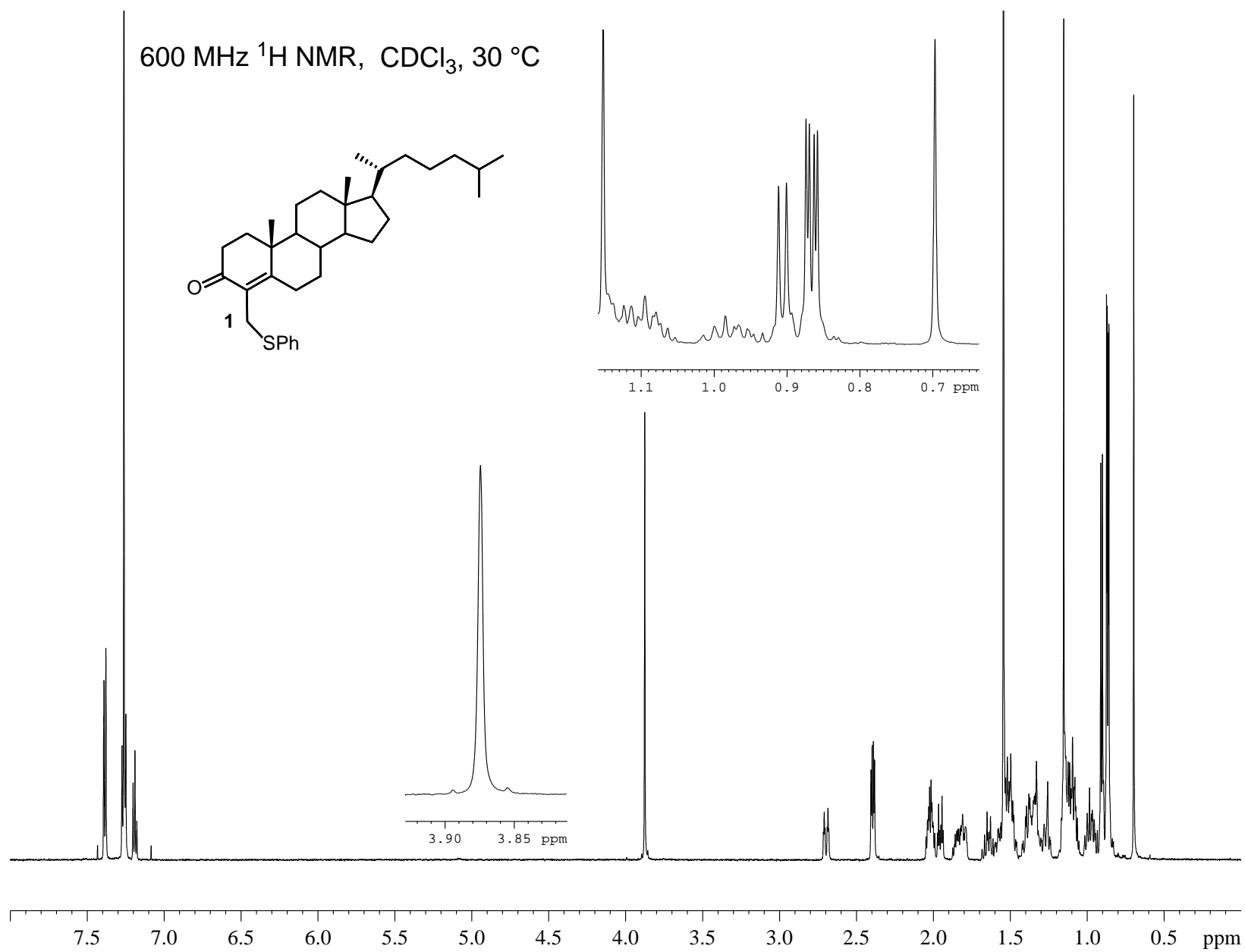
800 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



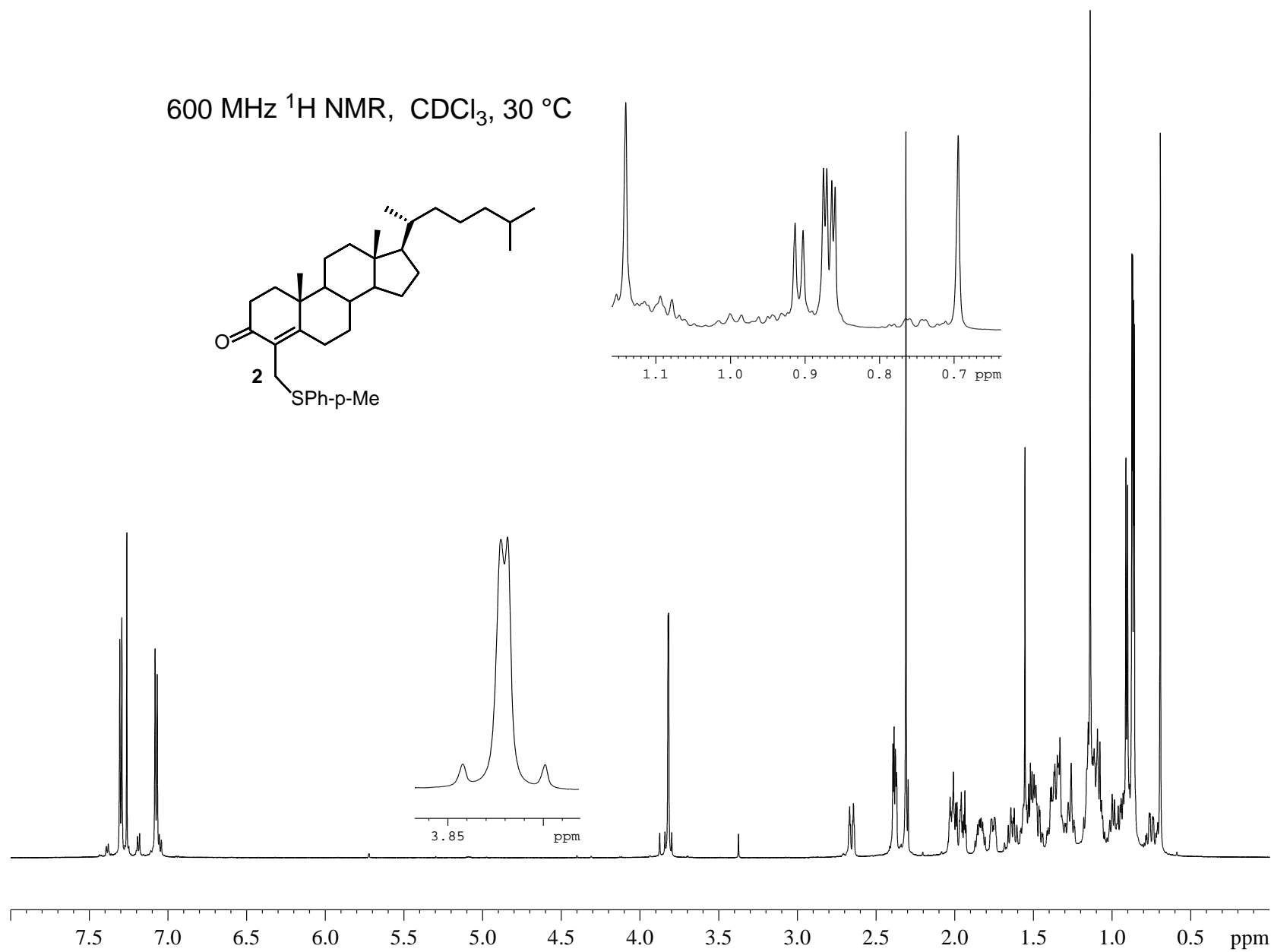
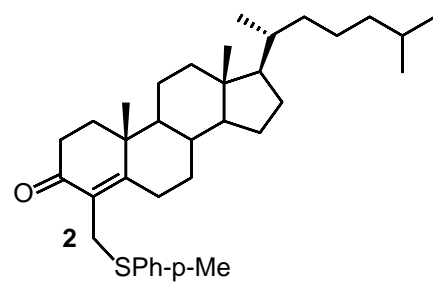
89



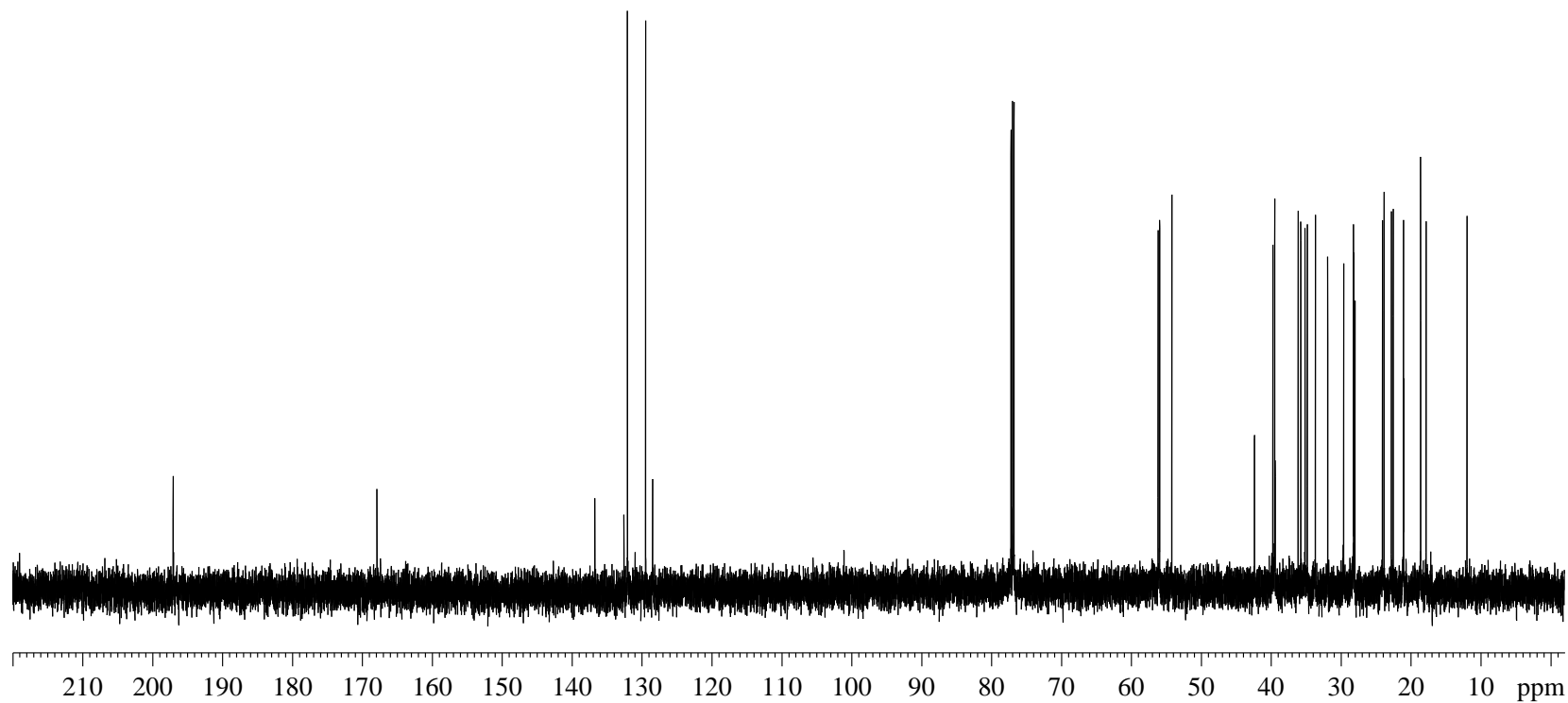
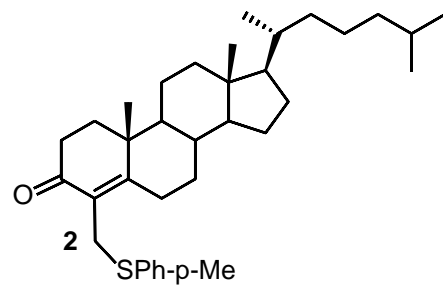




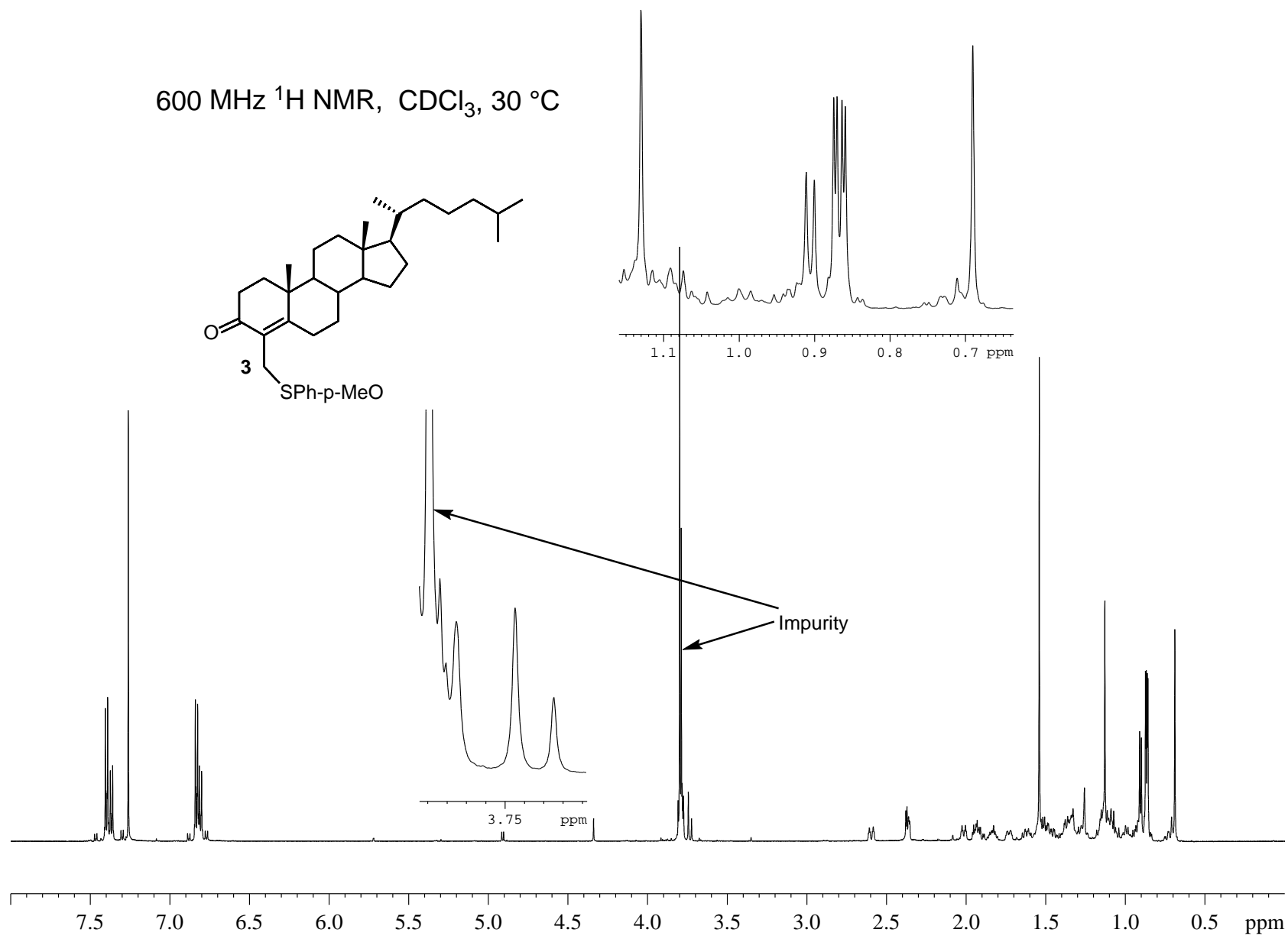
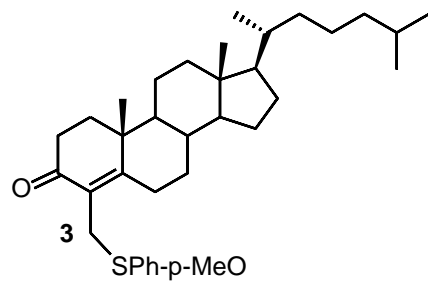
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



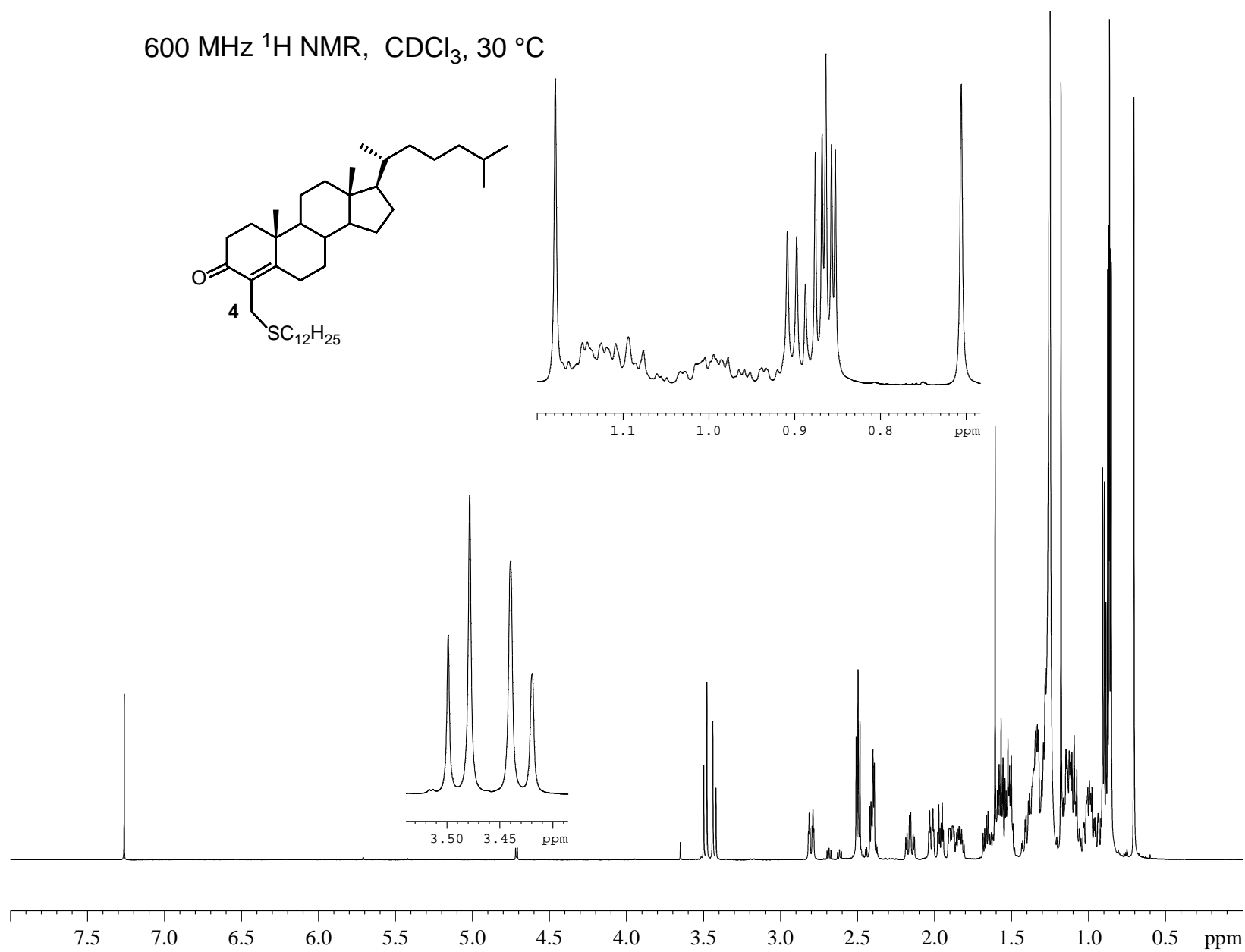
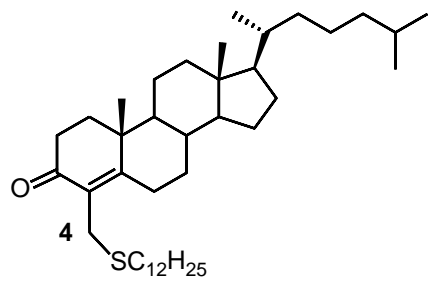
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



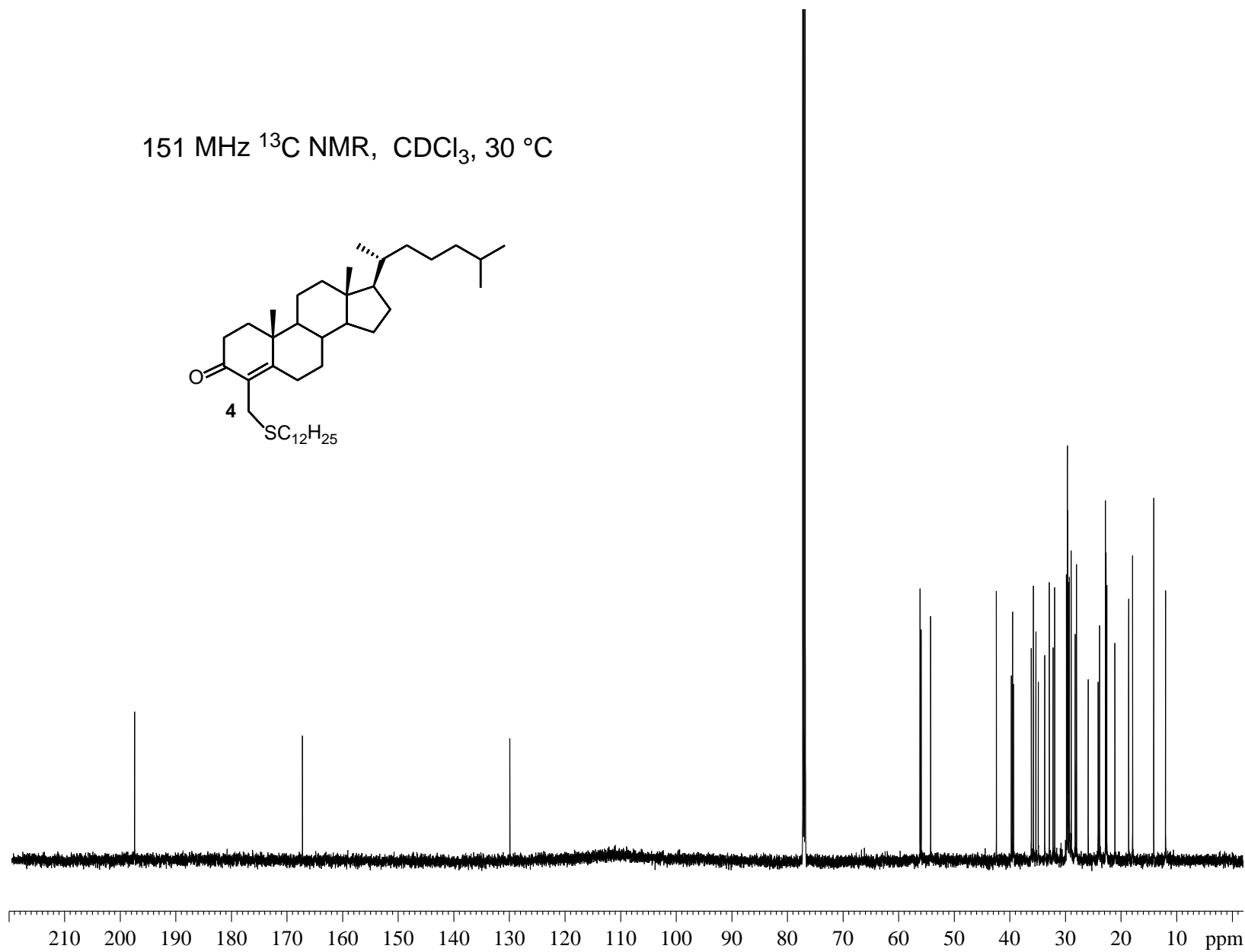
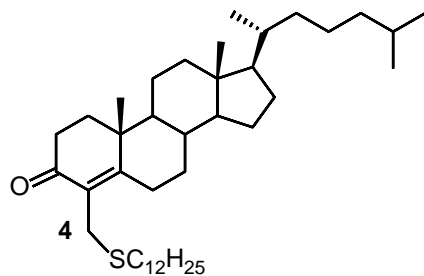
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

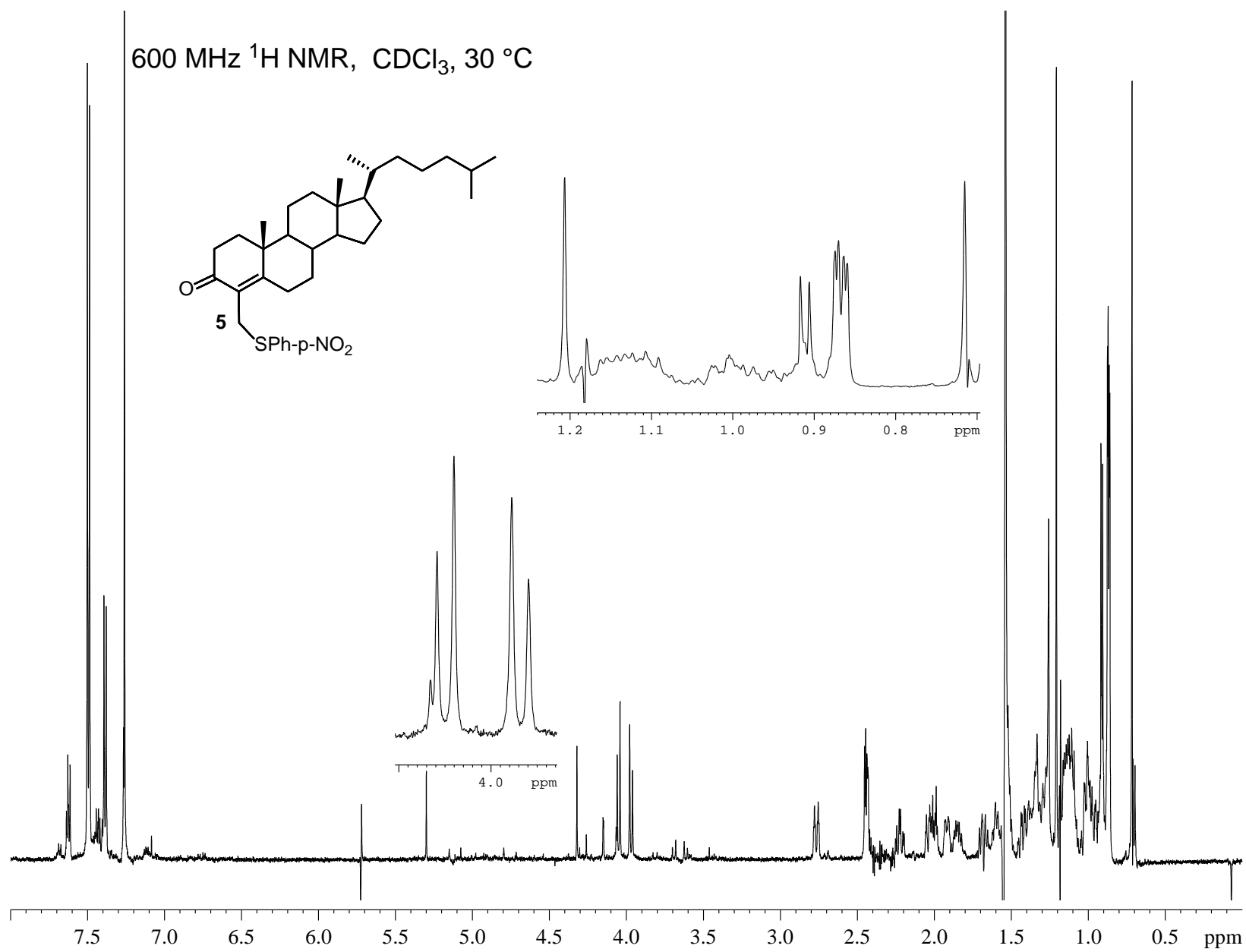


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

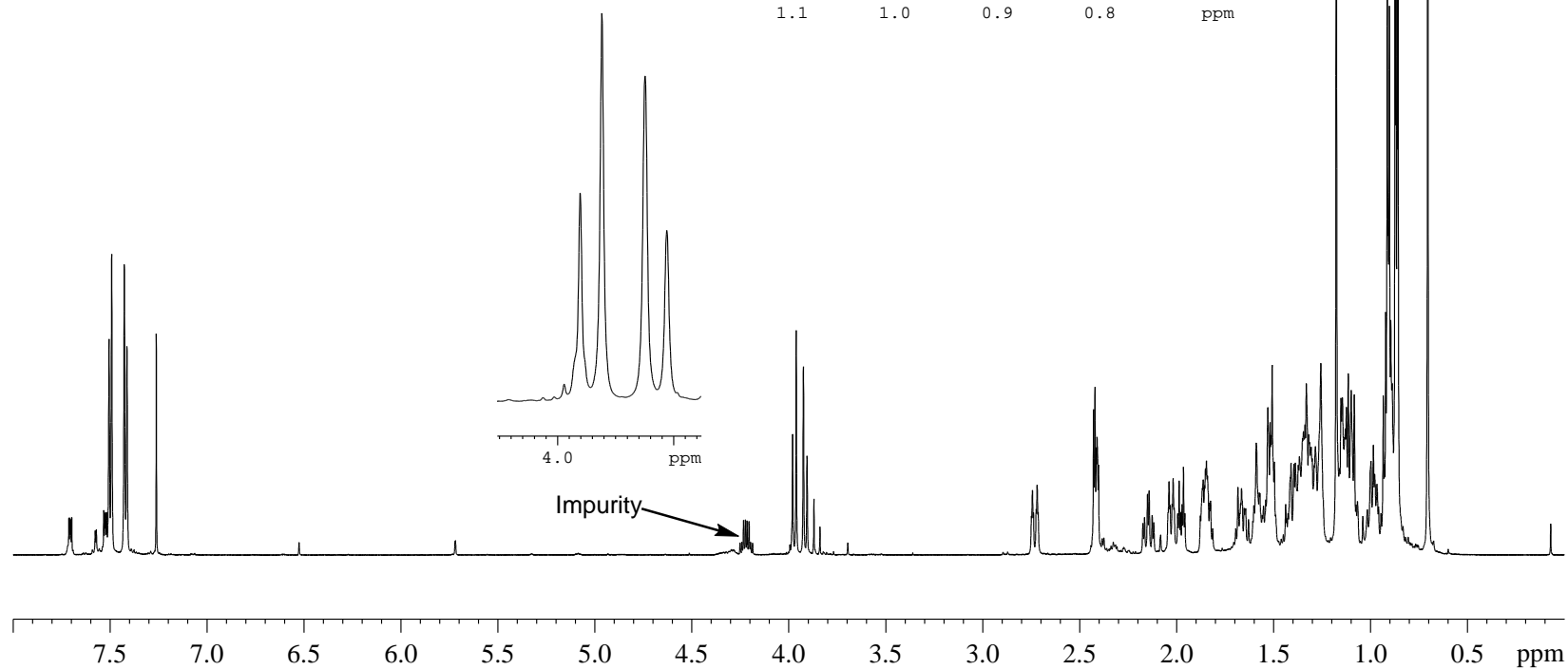
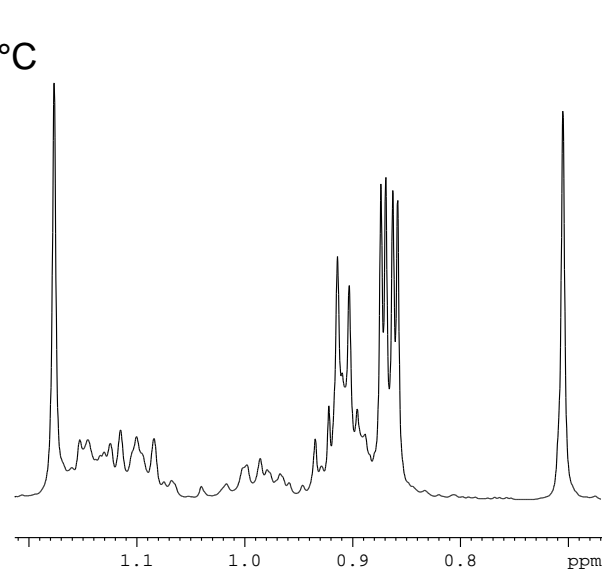
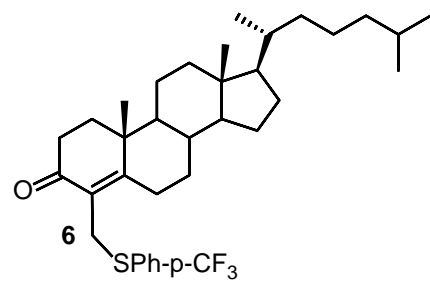


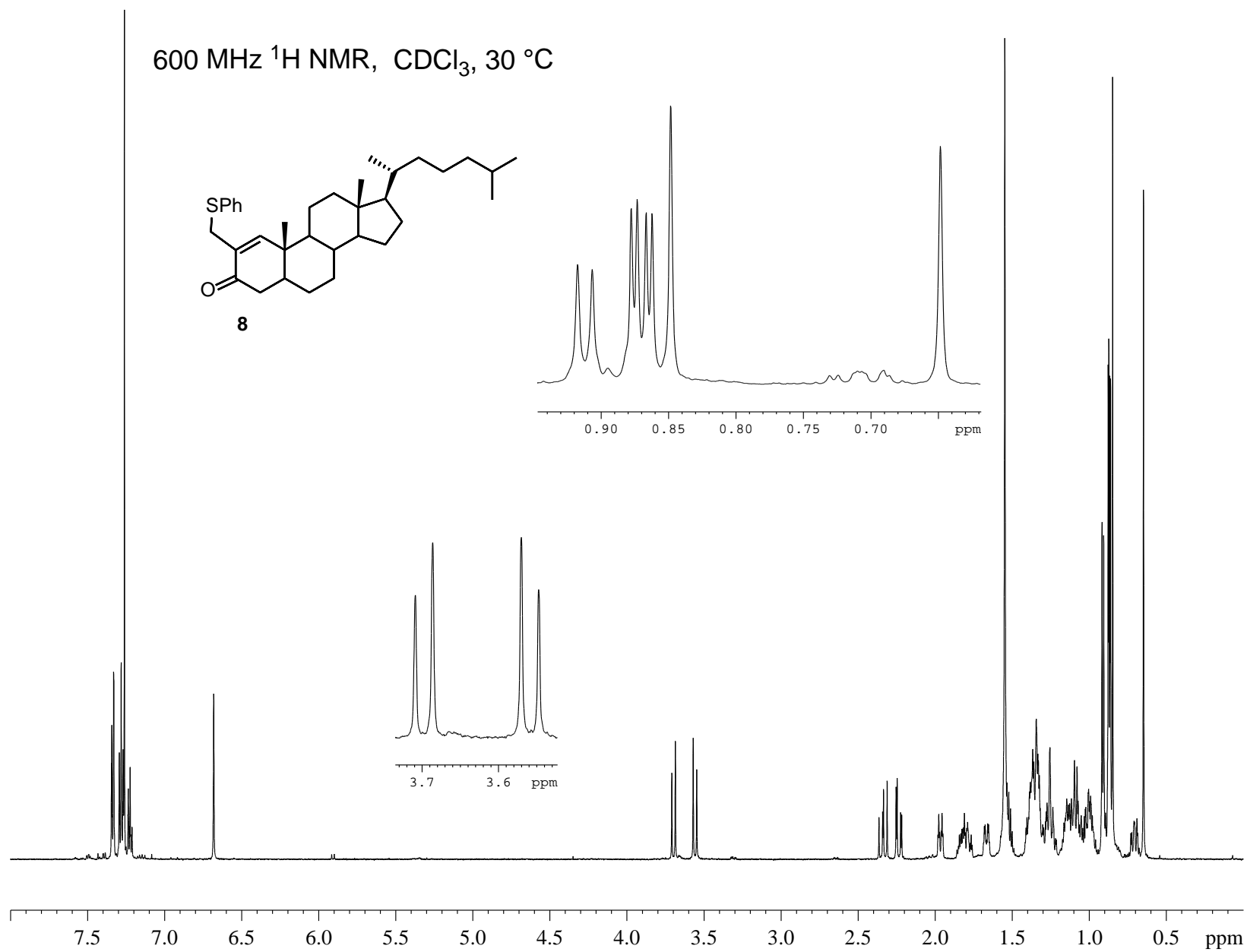
151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$



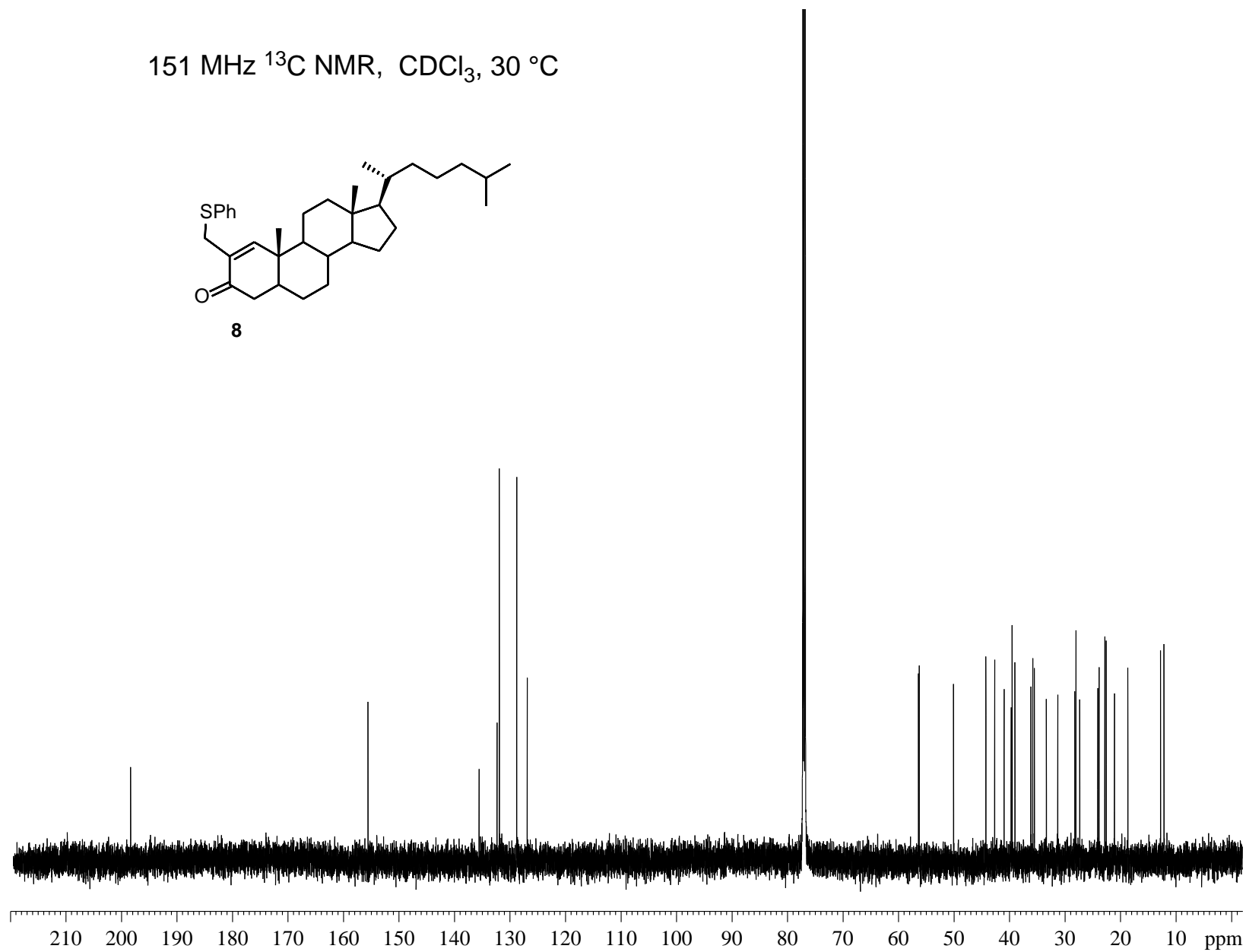
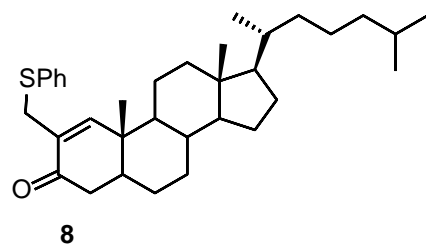


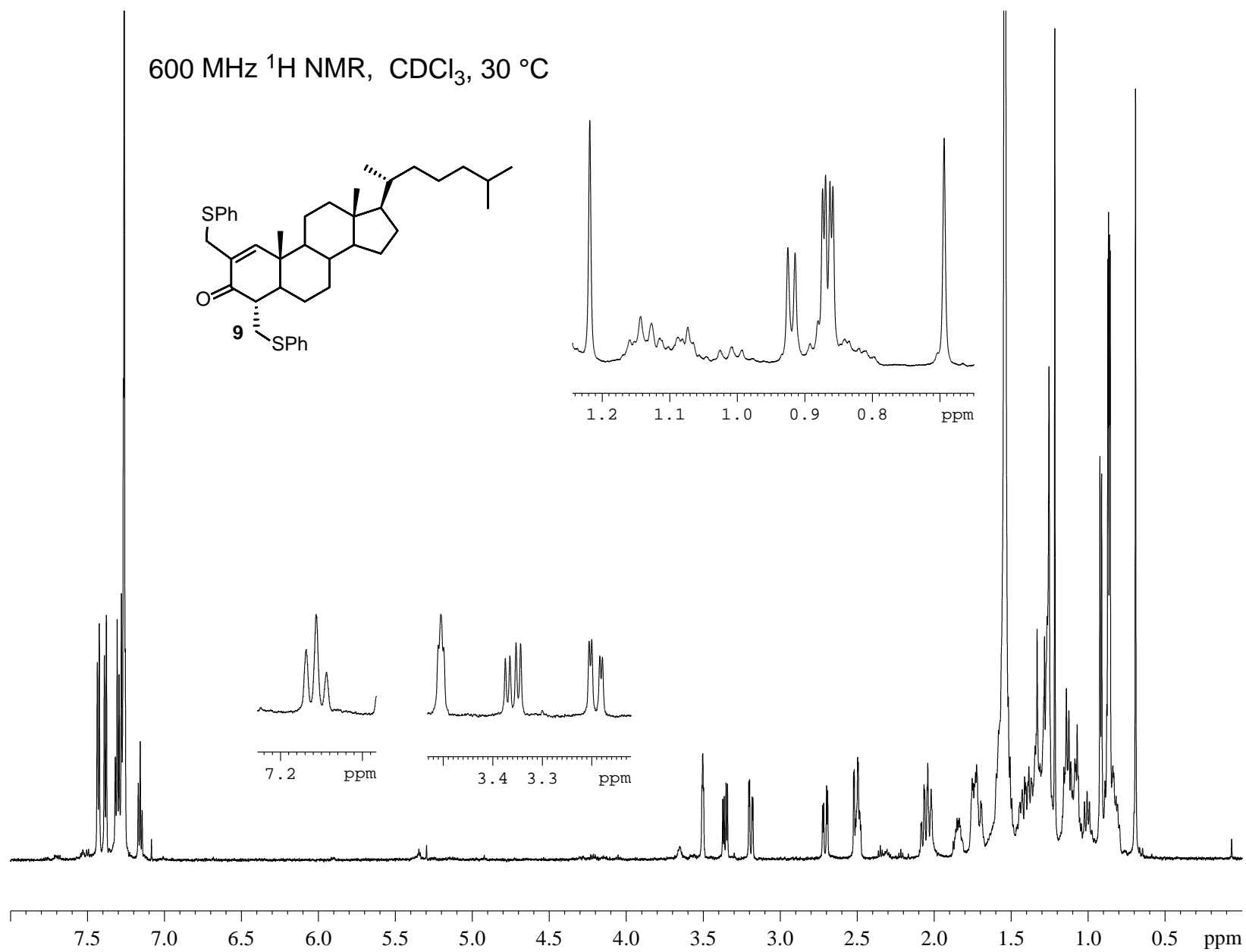
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



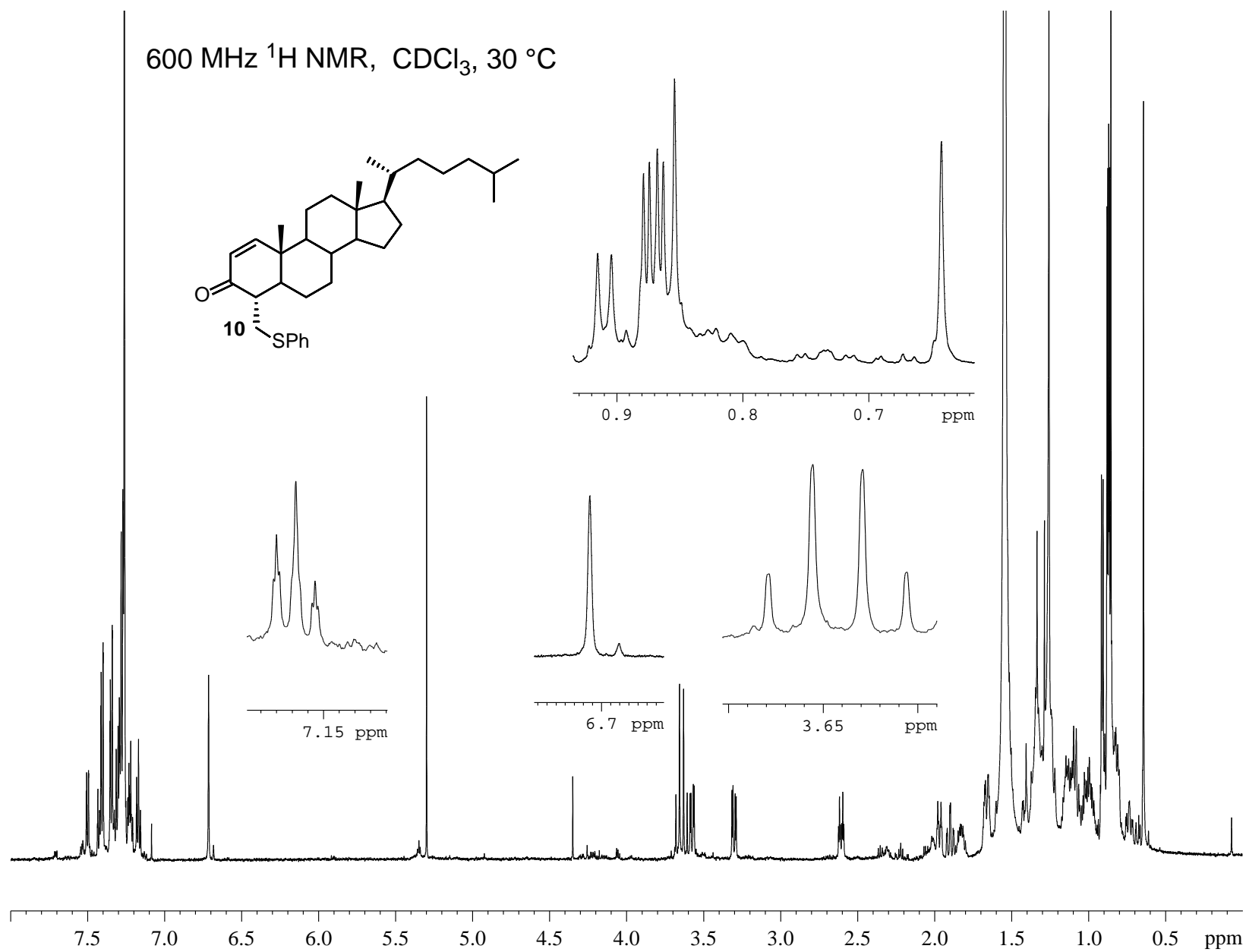
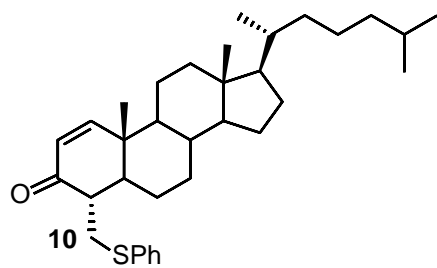


151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$

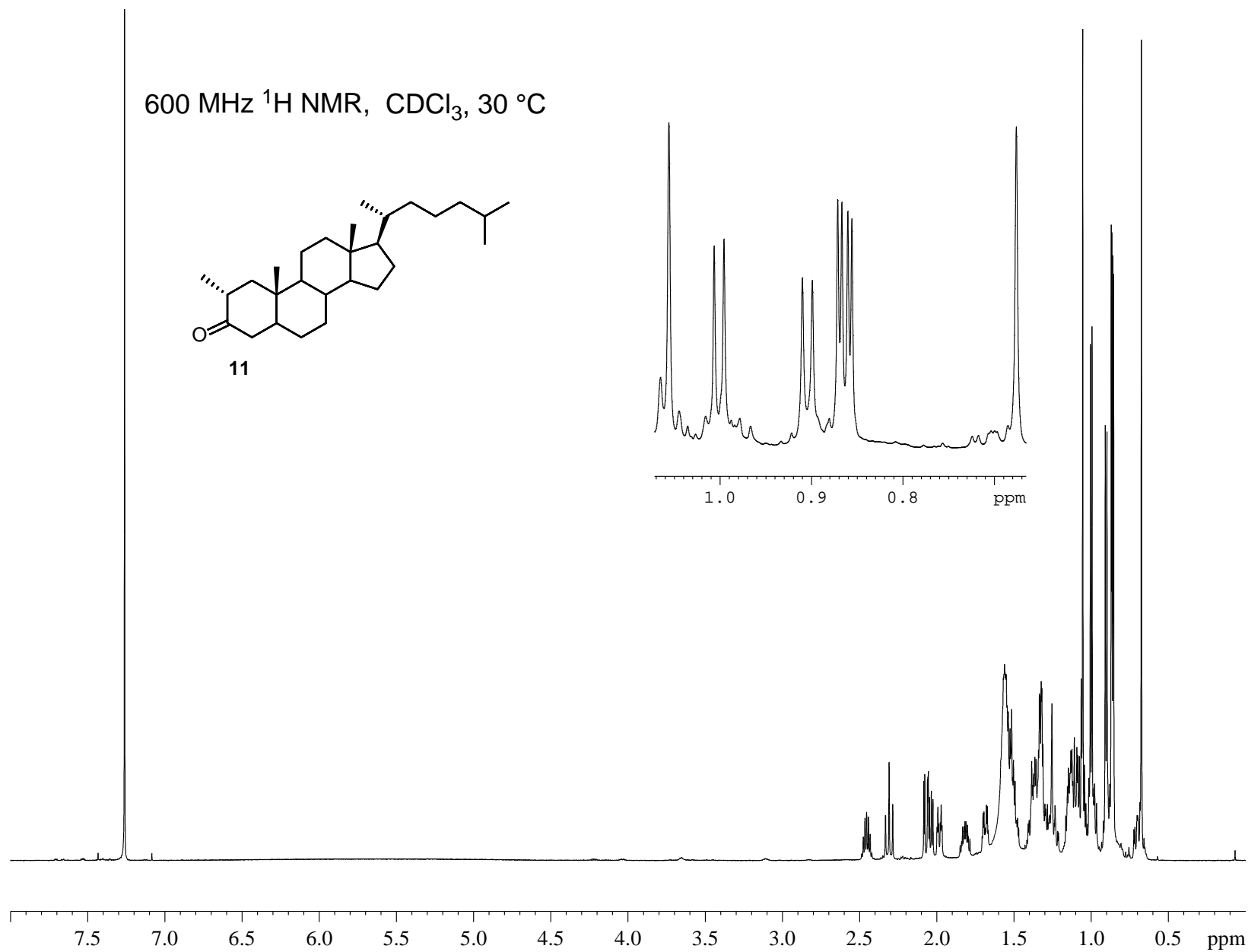
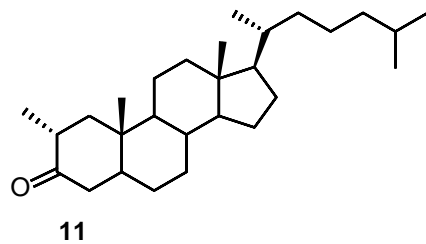




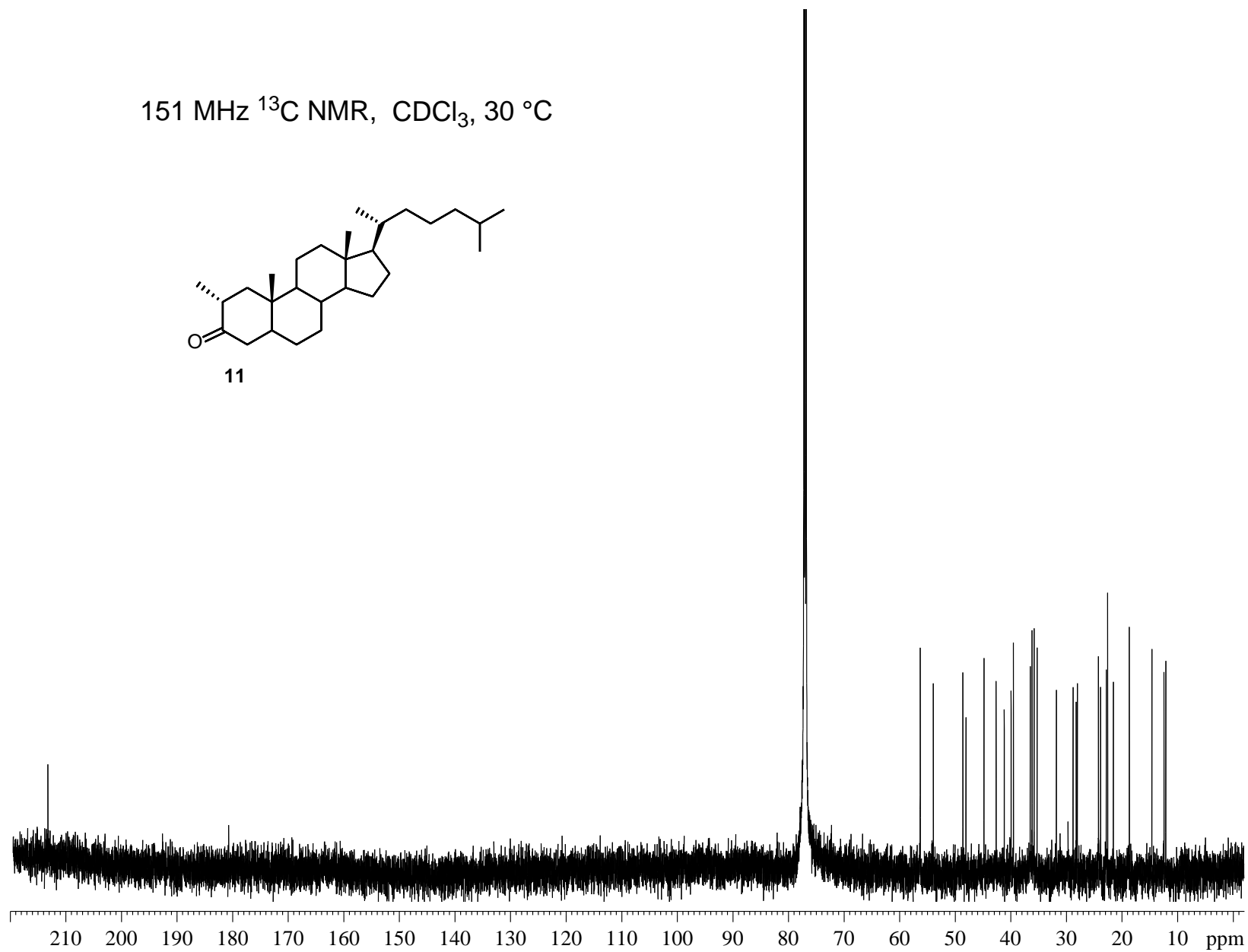
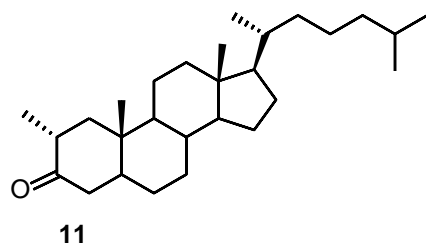
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

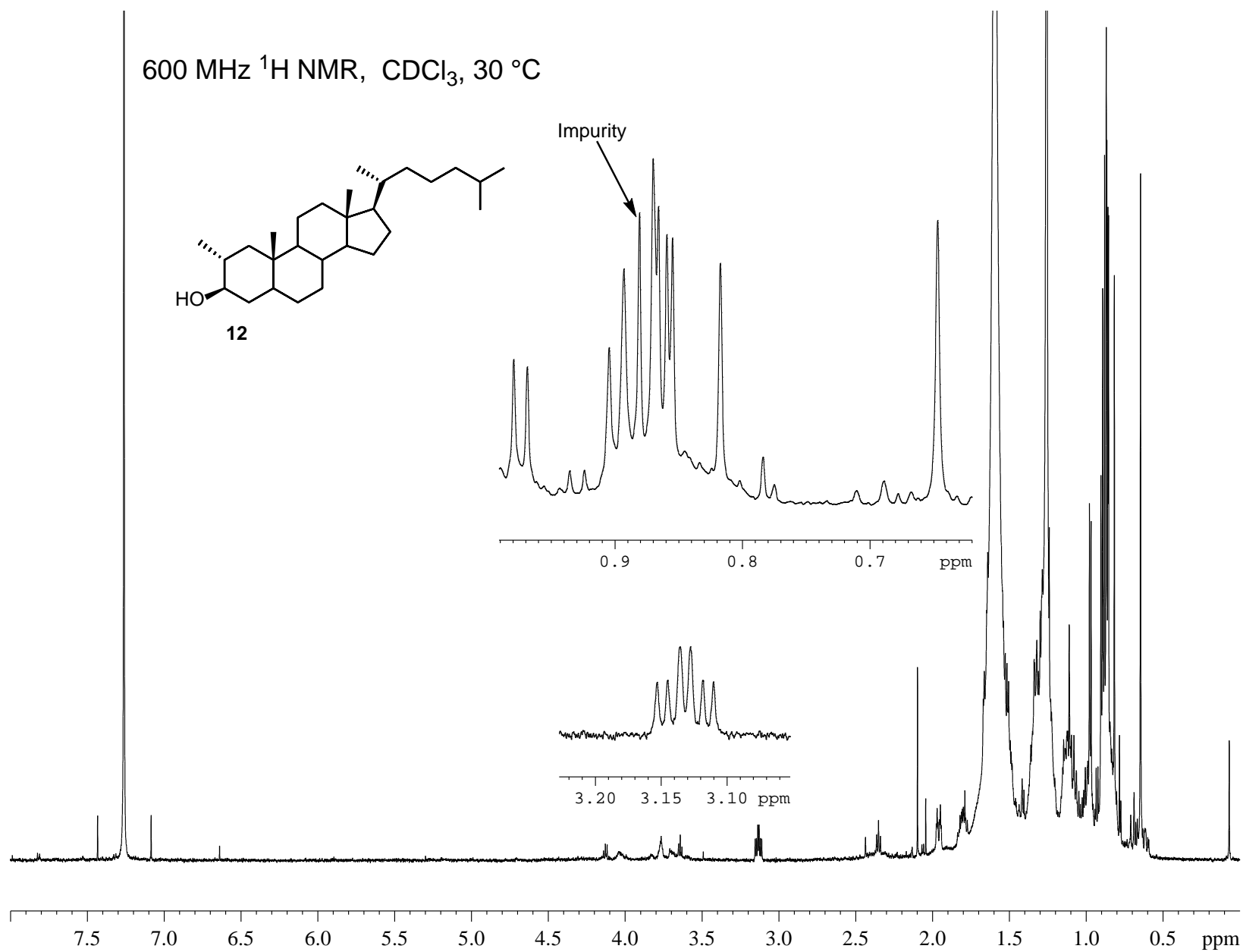


600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$

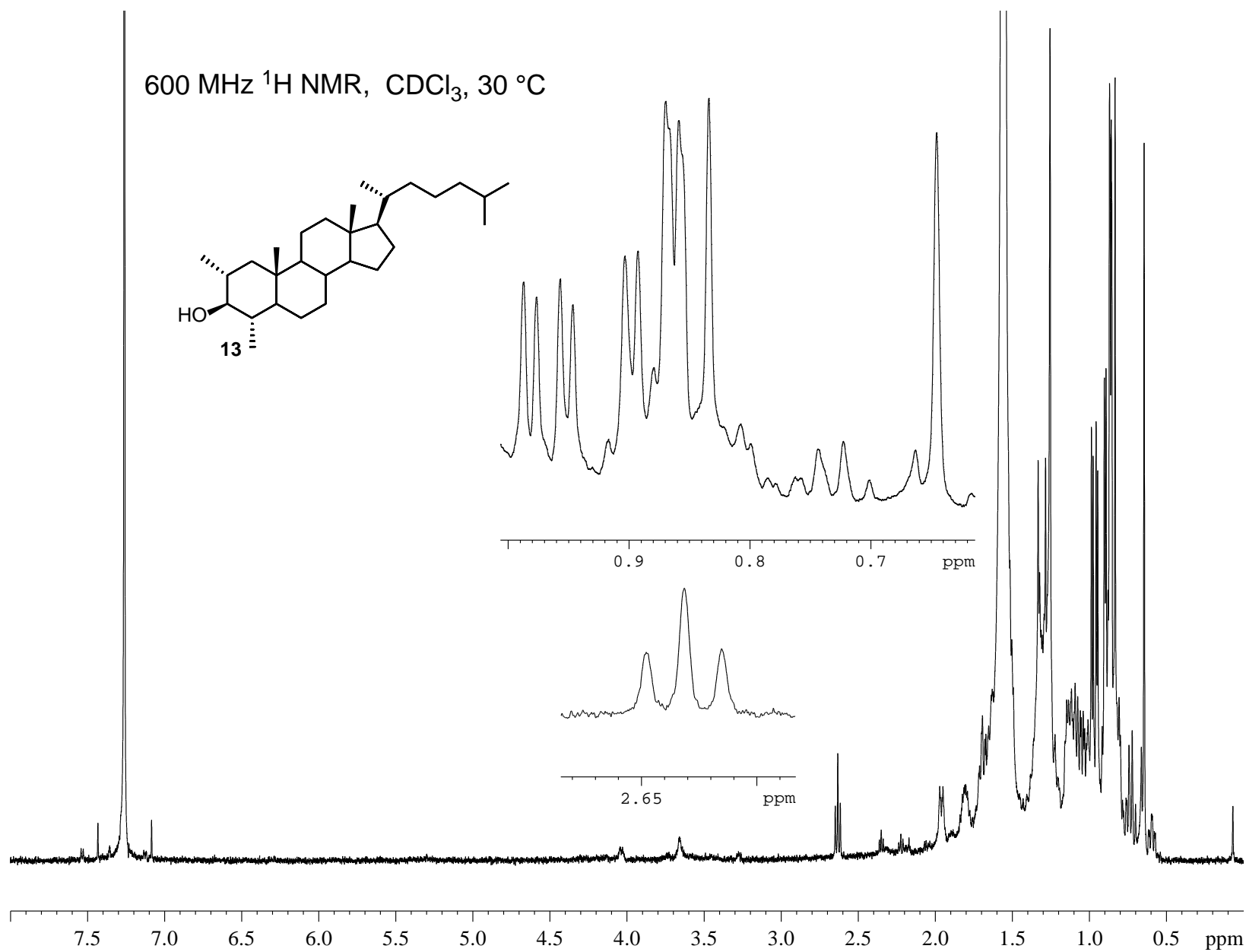
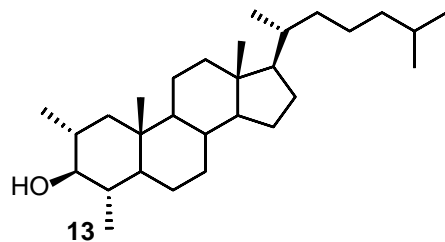


151 MHz ^{13}C NMR, CDCl_3 , 30 $^\circ\text{C}$

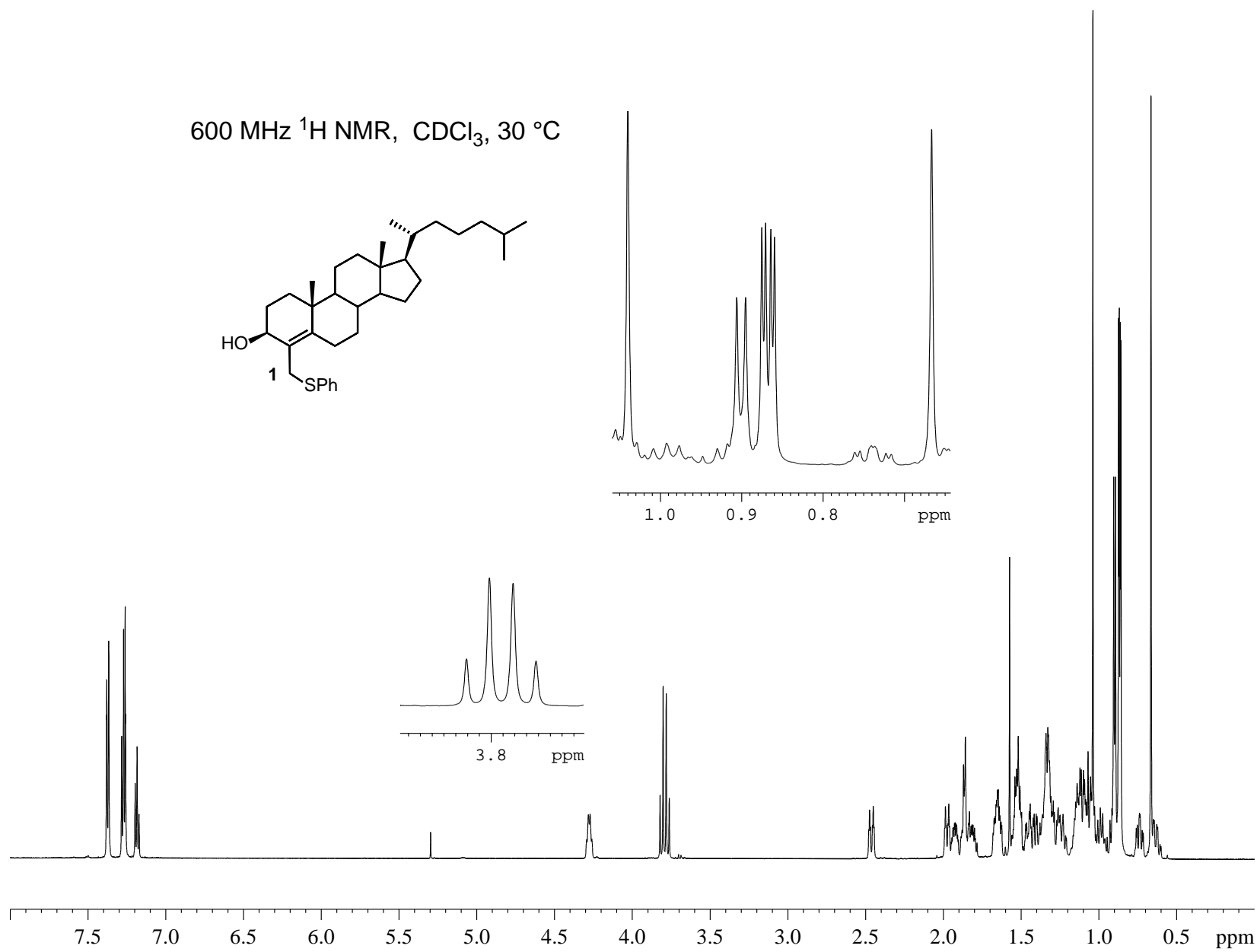
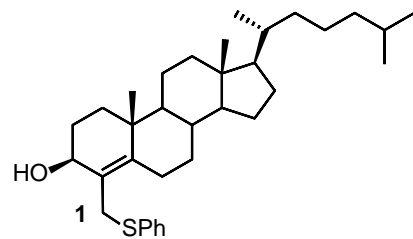




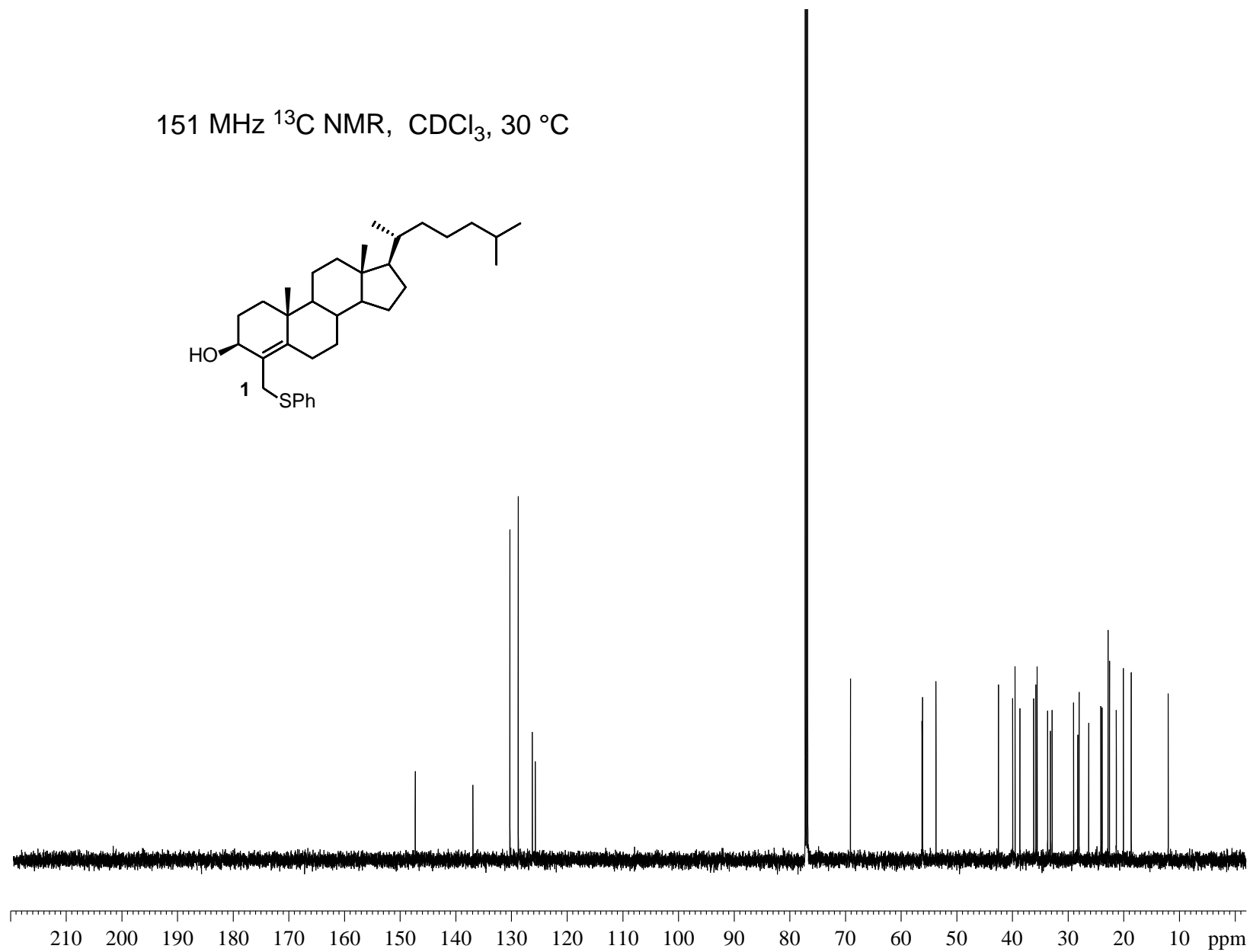
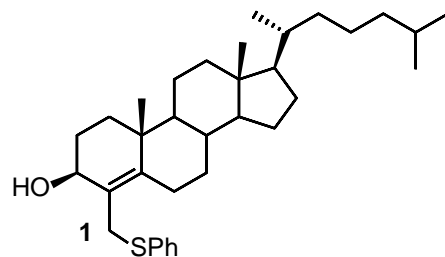
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



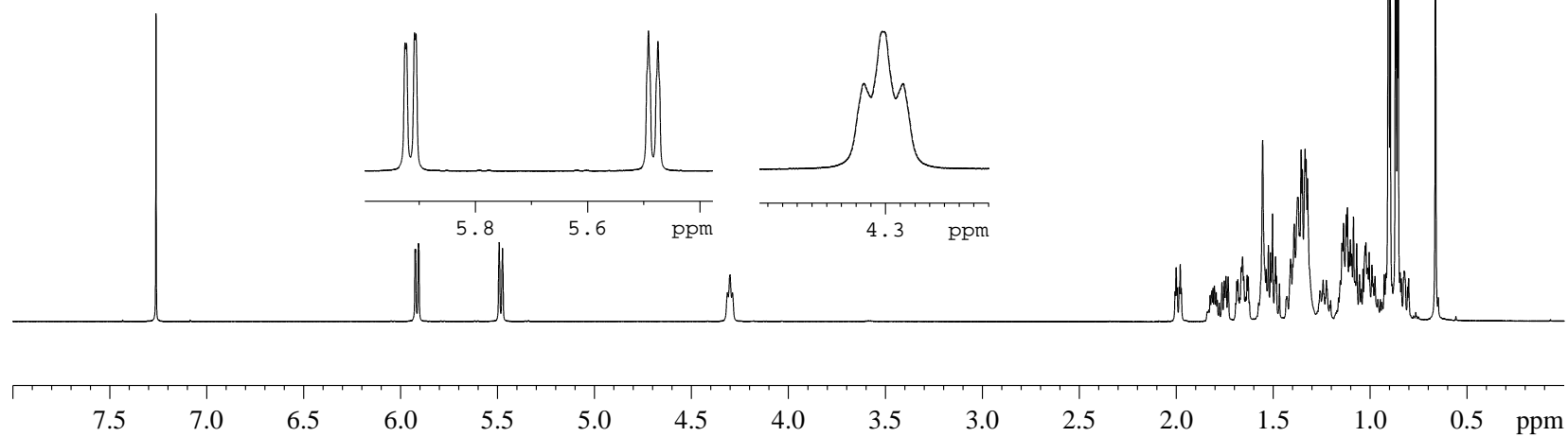
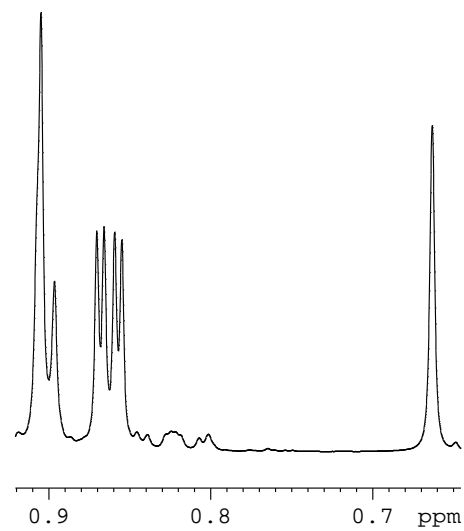
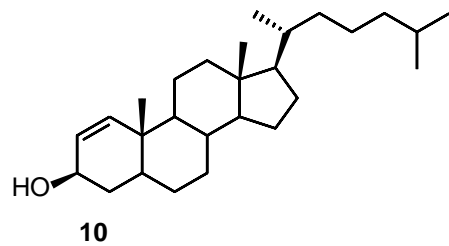
600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



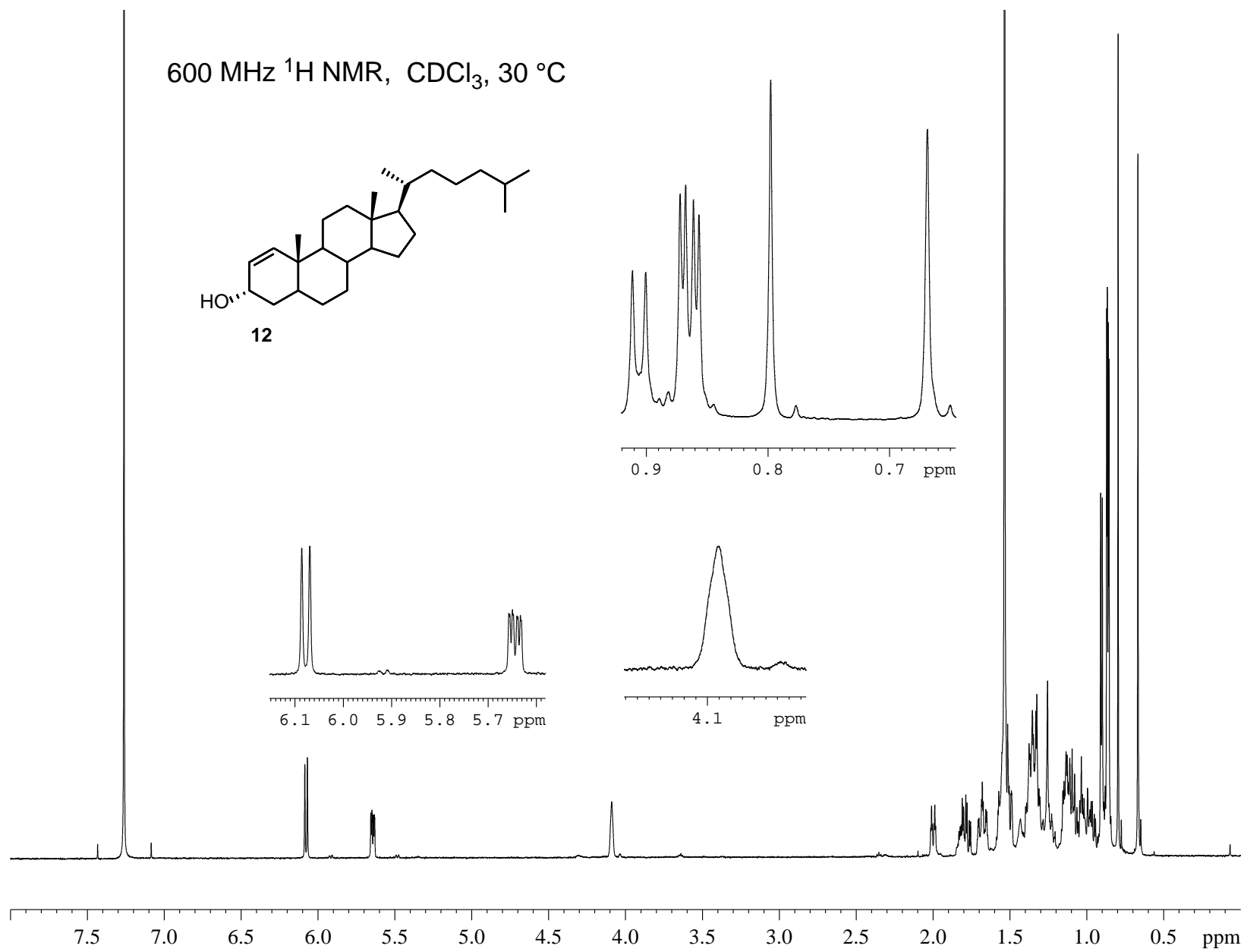
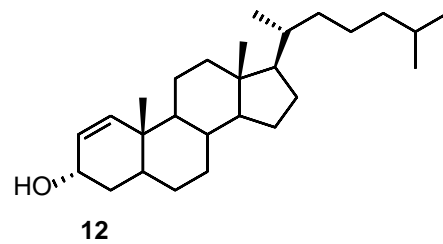
151 MHz ^{13}C NMR, CDCl_3 , 30 $^{\circ}\text{C}$



600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



600 MHz ^1H NMR, CDCl_3 , 30 $^\circ\text{C}$



Vita
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08/11-Present Ph.D. in Organic Chemistry of Natural Products, Expected: December 2017

**FLORIDA INTERNATIONAL UNIVERSITY
(Miami, FL)**

01/05-04/09 Bachelor of Science in Chemistry, 2009

**RELEVANT
EXPERIENCE**

**STATE UNIVERSITY OF NEW YORK-COLLEGE OF ENVIRONMENTAL
SCIENCE & FORESTRY (Syracuse, NY)**

08/11 – 08/15 Graduate Research Assistant (Organic Chemistry of Natural Products)

01/12 – 12/17 Graduate Teaching Assistant (General Chemistry-Organic Chemistry 3)

**UNITED STATES DEPARTMENT OF AGRICULTURE/
AGRICULTURAL RESEARCH SERVICE-SHRs (Miami, FL)**

07/09 – 07/11 Chemist (Chemistry/Entomology)

07/08 – 07/09 Biological Science Aid (Plant Sciences)

05/06 – 07/08 Physical Science Aid (Chemistry& Hydrology/Agronomy)

AWARDS

John A. Meyer Graduate Fellowship (2017).

Syracuse Research Corporation Graduate Student Fellowship (2015-2016)

Faculty of Chemistry Teaching Assistant Award (2014, 2016)

**TECHNICAL
SKILLS**

Organic synthesis, Steroid chemistry, Chromatography, HPLC, GC-MS, IR, NMR,
Microsoft Office (Word, Excel, PowerPoint), ChemStation, ChromQuest,
OMNIC, TOPSPIN (Bruker)

AFFILIATIONS

American Chemical Society member, US Army veteran

**PUBLICATIONS/
PRESENTATIONS**

Joseph, R., Giner, J.-L., *The 41st Northeast Regional Meeting of the American
Chemical Society*, Binghamton, NY, October **2016**.

Joseph, R., Giner, J.-L., *250th American Chemical Society National Meeting &
Exposition*, Boston, MA, August **2015**.

Giner, J.-L., Joseph, R., Berry, D., Ceballos, H., Wikfors, G. H., Hassett, R. P., "Diving
Deeper with Marine Sterols", *Chem. Listy* **2014**, *108*, 1092.

Giner, J.-L., Joseph, R., Wikfors, G. H., Hassett, R. P. *International Congress of Natural
Products Research 2012*, New York, NY, August **2012**.

Joseph, R., Reed, S.T., Jayachandran, K., Clark-Cuadrado, C., Dunn, C.B. *Agriculture,
Ecosystems and Environment* **2010**, *138*, 181-188.